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THE INFLUENCE OF INSULIN ON THE MOTILITY OF THE URINARY BLADDER (Dog)

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In 1924 Bulatao and Carlson reported an increase in gastric motility in normal fasting dogs as a result of injection of insulin and an inhibition of this activity with subsequent intravenous injection of dextrose. Quigley, Johnson and Solomon (1929) have noted a similar effect of insulin on the stomach of the normal fasting human subject. Quigley and Solomon (1930) extended their investigations to the human duodenum and to the dog's colon and in both cases reported that injection of insulin increased the motility of these organs. In view of these findings, which indicate that insulin has a more or less general and similar effect on the motility of the entire gastro-intestinal tract, it was decided to perform experiments on another smooth muscle viscus, the urinary bladder, as a step toward determining whether or not insulin causes an increase in activity of smooth muscle in general.

Methods. Normal unanesthetized female dogs were used throughout. The animals were trained to lie still on comfortable pads for periods of four hours. There was an average period of 16 hours between the last feeding and the start of the experiments. Insulin was injected subcutaneously in doses of 5 to 60 units, and dextrose was injected intravenously in 50 per cent solution. The usual experimental procedure was to take a control tracing of approximately 15 minutes' duration at the start of each experiment. Without interruption of the record insulin was injected, and the tracing was continued for about 4 hours. Control experiments lasting 4 hours were run at intervals on each dog.

Two methods of obtaining tracings of urinary bladder motility were tried. In the first a small condom balloon on a no. 10 hard rubber catheter was attached to a water manometer. The balloon and catheter were placed in 0.1 per cent $HgCl_2$ for several minutes and then washed with

sterile water. The vulva was swabbed with mercurochrome, and the balloon was inserted as aseptically as possible through the external urethral orifice into the bladder. The inflation pressure averaged 7.5 cm. of water.

The second method was similar in principle to that of Mosso and Pellacani (1882) in that a fluid system was used for recording volume changes. The fluid used consisted of one part of hexylresorcinol solution (1:1000) and three parts of 0.9 per cent NaCl. This fluid did not give rise to cystitis or urethritis, as determined at autopsy. The apparatus consisted of a calibrated pressure bottle connected with a manometer on the one hand and through a no. 14 hard rubber catheter with the interior of the urinary bladder on the other. The bulb on the pressure bottle was used to fill the system with fluid, after which a measured amount of fluid could be run into the urinary bladder. Depending on the size of the dog, the

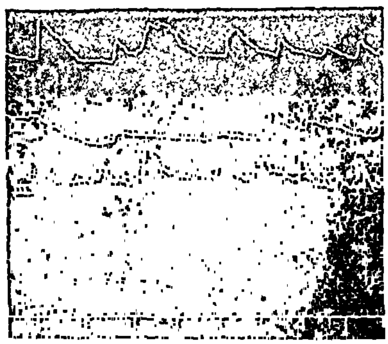

Fig. 1. Tracing of normal urinary bladder motility of 3 dogs by the fluid method, showing a maximum degree of normal motility.

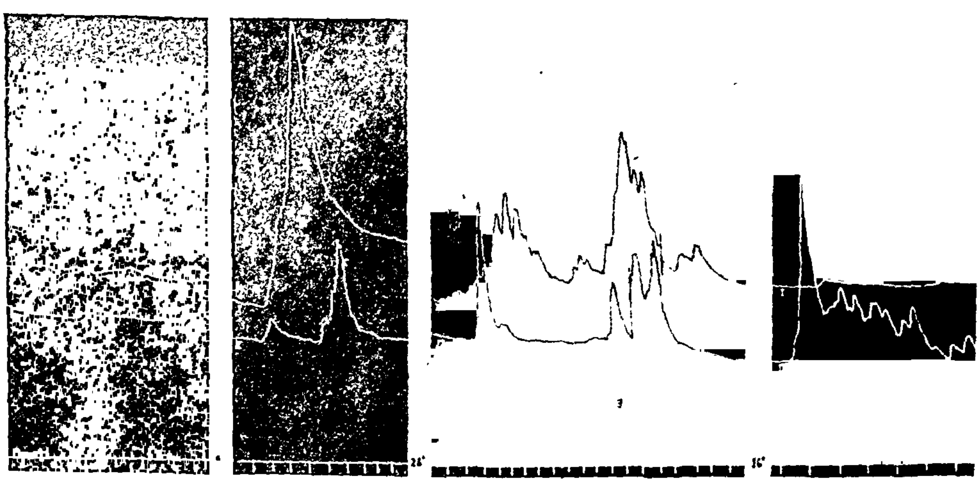

Fig. 2. Tracing showing effect of insulin on urinary bladder motility of 2 dogs by the fluid method. At *1* dog I was given 30 units of insulin subcutaneously. At *2* dog II was given 24 units of insulin. At *3* dog I was given 40 cc. of a 50 per cent dextrose solution intravenously.

quantity of fluid in the urinary bladder at the beginning of the experiments varied from 35 to 75 cc. with intra-vesical pressures ranging from 5 to 12 cm. of water.

Operations. Perineotomy was performed on each dog, the vulvar slit being extended dorsally, in order to expose the external urethral

orifice and thus facilitate the passage of the catheter. When the fluid method was used on a dog, a second operation was performed at the end of a long series of experiments. The purpose of this operation was to place a condom balloon attached to a gold-plated cannula in the abdominal cavity. This operation made it possible to take control tracings of intra-abdominal pressure simultaneous with tracings of urinary bladder motility on the unanesthetized dog. A low rectus incision was made aseptically under ether anesthesia. The sterile cannula with the balloon attached was put into the abdominal cavity through this incision, and the free end of the cannula was brought out through the ventral mid-line, as low as practicable, by way of a second small opening made just large enough to allow the cannula stem to pass through. A flange on the cannula was brought

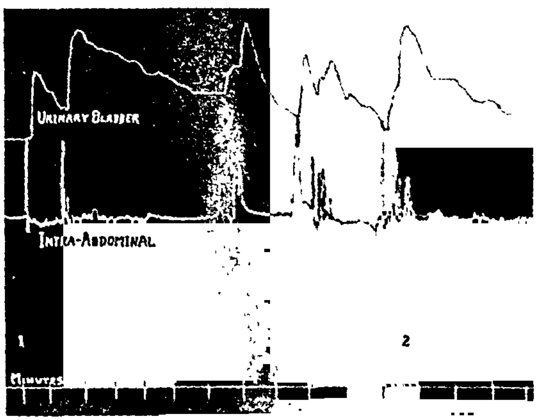

Fig. 3. Simultaneous tracings of urinary bladder motility and intra-abdominal pressure. The small excursions on the lower tracing are respiratory. There was hyperpnea during this period. *1*, 84 minutes after subcutaneous injection of 20 units of insulin. *2*, 15 grams dextrose intravenously.

to rest against the peritoneal surface. The omentum was drawn caudally so that it rested between the balloon and the intestines. The incision was closed and the animal was allowed to recover. The flange against the peritoneal surface kept the cannula and balloon from being pulled out. For tracings the extra-abdominal end of the cannula was connected with a water manometer and the intra-abdominal balloon was inflated. It was found that 5 to 6 days after the operation adhesions growing around the intra-abdominal balloon interfered with its inflation. Therefore control experiments on intra-abdominal pressure were run only at the end of a series of experiments.

Results. *By the balloon method.* The balloon method was used in 28 experiments, including controls, on 3 dogs. Judgment of the degree of motility was based on the frequency and height of the contractions. A

rise in tone was considered to have occurred when the writing-point rose from the normal level and did not return within 10 minutes. According to these standards the different control tracings showed varying degrees of motility without any appreciable spontaneous increase in motility or tone. After insulin an appreciable increase in tone as well as in motility was observed in several experiments. The increase in urinary bladder motility following insulin usually began within an hour after injection

TABLE 1

Motility and tonus of the urinary bladder of the dog by the balloon method

The number of experiments falling in each of the four categories of degree of motility and tone is given by figures in the respective columns.

DEGREE OF ACTIVITY	15 CONTROL EXPERIMENTS		13 INSULIN INJECTIONS, 5–30 UNITS*	
	Motility	Tone	Motility	Tone
+	12	11	4	8
++	3	4	6	3
+++	0	0	3	2
++++	0	0	0	0

* Usual dosage 20 units.
+ normal.
++ slight increase.
+++ moderate increase.
++++ marked increase.

TABLE 2

Motility and tonus of the urinary bladder of the dog by the fluid method

For explanation see table 1.

DEGREE OF ACTIVITY	90 CONTROL EXPERIMENTS		90 INSULIN INJECTIONS, 20–60 UNITS	
	Motility	Tone	Motility	Tone
+	64	73	7	11
++	22	14	14	9
+++	3	1	22	22
++++	1	2	47	48

and persisted until the end of the experiment. The rise in tone usually occurred about 2 hours after injection and persisted until the end of the experiment. Table 1 compares the results of insulin injections and controls.

By the fluid method. A total of 180 experiments half of which were controls and half experiments in which insulin was injected were done on 9 dogs. Periods of marked increase in motility or tone of the urinary bladder during the course of the control experiments were only rarely

observed. When insulin was given, however, there usually followed a marked increase in motility and rise in tone. The recorded changes after insulin, when the fluid method was used, were similar to but more marked than the changes recorded by the balloon method. Insulin convulsions, when they occurred, were always accompanied by micturition. In several experiments the activity of the bladder increased to such a degree that micturition occurred unaccompanied by convulsions or struggling. Since micturition usually necessitated terminating an experiment, an attempt was made to avoid convulsions by regulating the dose of insulin. In this series of 9 dogs the dose ranged from 20 to 60 units.

TABLE 3

Effect of dextrose on motility and tonus of the urinary bladder following insulin injection

The number of experiments falling in each of the six categories of results is given by figures in the respective columns.

RESULT	52 INTRAVENOUS INJECTIONS OF DEXTROSE	
	Motility	Tone
No effect	5	5
–	2	3
– –	5	6
– – –	10	10
– – – –	20	18
+++++	10 (?)	10

– slight diminution in activity.
– – moderate diminution in activity.
– – – marked diminution in activity.
– – – – return to normal.
+++++ further increase in activity over that following insulin.
(?) The increased activity following dextrose injection seemed to consist of a rise in tone although an increase in motility could not definitely be ruled out.

In none of the experiments in which intra-abdominal pressure was recorded was there any direct relationship between intra-abdominal pressure and the recorded motility of the urinary bladder. In two dogs the intra-abdominal balloons registered slight decreases in pressure more or less synchronous with bladder contractions. This might be expected since when the urinary bladder contracts and forces fluid out, the content of the abdominal cavity is diminished. On the other hand, such a fall in intra-abdominal pressure may have been adventitious, due to changes imposed on the balloon by intestinal motility synchronous with changes in bladder motility.

Effect of dextrose injection following insulin. In 52 experiments 10 to 15 grams of dextrose in 50 per cent solution were injected intravenously

when the urinary bladder activity was prolonged and at its height following insulin. The results of dextrose administration were variable. In the earlier experiments dextrose seemed to inhibit contractions consistently and to cause a decrease in tone to normal. There then usually followed a quiescent period lasting from 15 minutes to an hour, hyperactivity usually returning if the experiment was continued a sufficient length of time. Later experiments did not show such a marked and consistent effect of injection of dextrose. The impression was gathered, however, that individual contractions tended to be inhibited more than tone. In those experiments in which there was a balloon in the abdominal cavity, dextrose injection frequently brought about a rise in urinary bladder tone over that already present following insulin. The mechanism of the rise was not clear. Evidence of ascites was noted in some of these experiments, the abdominal wall being distended. The tracings of intra-abdominal pressure, however, showed no increases corresponding to the increases in bladder tone. This indicates that the mechanism was not a rise in intra-abdominal pressure due to the ascites. It is possible that the injection of dextrose intravenously in some instances caused a rapid diuresis which led to the rise in tone.

Discussion. Although the statement is frequently made that the normal motility of the dog's urinary bladder is very slight, 7 of the 9 dogs used here showed detectable motility normally. Elliott (1907) makes the generalization that "automatic rhythmic contractions practically do not occur in other bladders than those which possess inhibitory nerves." Yet Mosso and Pellacanis' (1882) tracings show that some dogs have considerable rhythmic activity. In this work normal rhythmic contractions seemed to be the rule rather than the exception.

The site of action of the insulin was not determined. Future experiments on the partially and totally denervated bladder may indicate the place of action of insulin in increasing the activity of the urinary bladder.

SUMMARY

1. Subcutaneous injection of insulin causes an increase in motility and tone of the unanesthetized female dog's urinary bladder.

2. Micturition with or without convulsions may occur at the height of the hyperactivity following insulin.

3. The recorded increases in bladder activity were not due to changes in intra-abdominal pressure.

4. The effect of dextrose administered intravenously on insulin hyperactivity is not constant, but inhibition is frequently noted.

5. In this series of dogs the majority showed a considerable degree of urinary bladder motility, prior to injection of insulin.

The writer wishes to express his appreciation to Dr. A. J. Carlson for facilities freely offered and for helpful guidance in this work, and also to Dr. M. M. Kunde for advice and assistance in technical difficulties.

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RELATION OF THE HYPOPHYSIS AND OVARIES TO EXPERIMENTALLY-INDUCED UTERINE BLEEDING IN MONKEYS

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The cyclical changes of the uterus are associated with concomitant changes in the ovary, and the ovarian function depends largely upon the secretion of the anterior lobe of the hypophysis (Smith, 1926; Smith and Engle, 1927, and Zondek and Aschheim, 1927), but the definition of menstruation, which is one of the most manifest symptoms of the cyclical changes in the uterus of the human and other primates, is not clear. However, it is recognized that the bleeding from the endometrium is the most important factor of menstruation. Corner (1927) has found in macaques that menstruation may proceed, at least for a limited period, without ovulation or the formation of a corpus luteum. Edgar Allen (1927, 1928b), Maddux (1930), and Morrell and associates (1930) have shown that when the follicular hormone (oestrin, folliculin, amniotin, etc.) is injected into spayed macaques, bleeding from the uterus results after the cessation of injections. Edgar Allen (1928a) found that the transplantation of the anterior lobe of the hypophysis in non-castrated immature macaques caused considerable growth in the genital tract and marked growth of the Graafian follicles. This led him to suppose that the development of the genital tract and the effects on the sex characteristics were secondary to changes in the ovary, for, as noted by him, these changes are similar to those obtained in normal and castrated monkeys from injections of ovarian hormone. The finding of Smith and Engle (1927) that anterior lobe implants were ineffective in castrated rats also supported this supposition of E. Allen.

Hartman, Firor, and Geiling (1930) were able to cause bleeding from the uterus in macaques by injection of extracts of the anterior hypophysis. In a series of experiments, they found evidence that the extract of the anterior hypophysis caused bleeding in hypophysectomized macaques while follicular hormone did not. This evidence with others led them to

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postulate the presence of a special anterior lobe hormone as the cause of bleeding, distinctly different from the follicle-stimulating hormone of Smith and Engle and the luteinizing hormone of Evans and Simpson (1928).

The object of the present work is to find out whether or not extracts of the anterior lobe of the hypophysis can cause external bleeding from the uterus like that found during normal menstruation as stated by Hartman and co-workers, and also to investigate further the relation of the ovaries to the phenomenon in question.

The animals used in this experiment were eight immature rhesus monkeys, weighing from 2400 to 3400 grams, and two mature rhesus monkeys. The immature animals were estimated to be between three and four years of age and had not passed through the first menstruation. The sheep hypophysis extract used was kindly made for us from whole hypophyses by Parke, Davis & Co. (See Bugbee, 1931.) One cubic centimeter of the extract corresponds to 0.25 gram of the fresh tissue of the hypophysis.

The first two experiments involved simply the administration of hypophysis extract to normal immature monkeys.

Macaque 69, weight 2475 grams, immature. Beginning February 19, 1931, 36 cc. of the hypophyseal extract were injected intraperitoneally over a period of 8 days. Increased reddening of the sexual skin became noticeable on the third day of the injections and persisted until the fourth day after the last injection when it began to fade. The swelling of the vulva began on the sixth day. Uterine bleeding, as manifested by external vaginal bleeding, began 8 days after the last injection and continued for 6 days. Microscopic bleeding, however, was apparent for 2 days more.

Macaque 70, weight 2810 grams, immature. Thirty-six cubic centimeters of the hypophyseal extract were injected over a period of 8 days. The reddening and swelling of the vulva were almost the same as seen in no. 69. The external bleeding began 7 days after the last injection and continued for 5 days. For 2 days before that and 3 days afterward erythrocytes were seen in the lavage microscopically.

These two experiments show that the injection of hypophyseal extracts can cause external bleeding which in amount and duration is similar to that found during normal menstruation. No gross bleeding was seen during the injections, but upon microscopic study of the lavage a few red blood cells were found on the second and third day. In my experience the number of red blood cells found at this time was no greater than those occasionally observed in normal untreated monkeys.

In the next three experiments injections were continued longer to see whether bleeding might occur during the injections.

Macaque 82, weight 3300 grams. Fifty-nine cubic centimeters of the extract were injected over a period of 17 days. Swelling of the vulva began on the fourth day and became very marked near the end of the period of injection, but no bleeding was seen.

Typical bleeding began 6 days after discontinuing the injections and lasted for 7

days. However, microscopic bleeding was observed for 1 day before and 9 days after the external bleeding. The color of the sexual skin and the swelling of the vulva began to regress soon after the injections were discontinued, and 1 day before the onset of bleeding they had practically disappeared.

Macaque 60, weight 3390 grams (mature?). This animal had menstruated only once since she was purchased in May 1930, namely, in October 1930 for 3 days. Fifty-nine cubic centimeters of the extract were injected over a period of 17 days beginning January 26, 1931. There was no bleeding during the injection, except for a few red blood cells which were seen microscopically on the second and third days. In this case also the external bleeding occurred 8 days after discontinuing the injections and lasted for 7 days, a day before that and 3 days after that microscopically. The swelling and reddening were similar to those found in no. 82.

Macaque 72, weight 3150 grams, immature. Forty-three cubic centimeters of the extract were injected over a period of 14 days. The reddening and much swelling were observed. Six days after stopping the injections the typical external bleeding began, but no bleeding was seen microscopically or macroscopically during the injections.

In all cases, therefore, where injections were continued for 14 or 17 days, the menstruation-like bleeding occurred after discontinuing the injections, in exactly the same manner as when injections were made for only 8 days.

The next three experiments were designed to show whether or not the bleeding may be induced at intervals shorter than the normal menstrual cycle.

Macaque 82, second experiment. Three weeks after the onset of the last experimental bleeding 18 cc. of the extract were injected again over a period of 7 days. External bleeding was detected 5 days after the last administration and lasted for 6 days. Microscopical bleeding was observed for 2 days after this period, but neither during the injection nor before the external bleeding. Swelling and reddening were observed in this case also.

Macaque 69, second experiment. In no. 69, following the last day of the bleeding, 30 cc. of the extract were injected over a period of 7 days. The swelling and reddening began on the third day and external bleeding on the seventh day after the last injection, 3 weeks after the onset of the preceding bleeding. Microscopical bleeding was seen the day before and for 2 days after that. Seven days after this second experimental bleeding the animal was injected for the third time, 21 cc. of the extract being given over a period of 8 days. External bleeding began, for the third time, 9 days after stopping the injection. On the second day of this menstruation both ovaries and the uterus were removed surgically.

Macaque 70, second experiment. Two days after cessation of the first experimental bleeding this animal was again injected for a period of 7 days with 25.5 cc. of the extract. Swelling and reddening began during the period of injection. Bleeding appeared 5 days after the last injection and lasted for 6 days. Beginning 10 days later the animal was injected with 21 cc. of the extract in 8 days, but no bleeding was observed either macroscopically or microscopically. Very slight swelling for the last 3 days and a little reddening for the last 2 days of the injection were observed, however.

These experiments show that experimental menstruation-like bleeding can be produced 2 or 3 times in the same macaque and at intervals shorter than the normal cycle, by the injection of an extract of the hypophysis.

Four experiments were next performed in which the hypophysis extract was administered to spayed monkeys.

Macaque 65, mature. Menstruation had been irregular. Bilateral oöphorectomy was performed January 28. On January 30 some red blood cells were observed in the lavage and on January 31 a small blood clot was found in the vagina. After that there was no bleeding. On February 26 the injection of the hypophyseal extract was begun and continued until March 5, the doses totalling 51.5 cc. At various times, both during and after the period of injections, a few red blood cells were seen microscopically in the washings; for instance, 1 or 2 in some fields under high power. This slight and irregular bleeding, though detectable microscopically, was insignificant when compared with that produced in non-castrated animals by the same extracts. Two weeks later 34 cc. of the extract were injected in the animal over a period of 8 days, but no bleeding was observed either macroscopically or microscopically, and the animal died of an unknown cause 8 days after the last injection. During these injections no swelling of the vulva was seen.

Macaque 68, weight 3390 grams, immature. Oöphorectomy was performed March 16. Beginning April 10, 30 cc. of the hypophyseal extract were injected over a period of 11 days, but neither macroscopical nor microscopical bleeding was observed either during or after the injections.

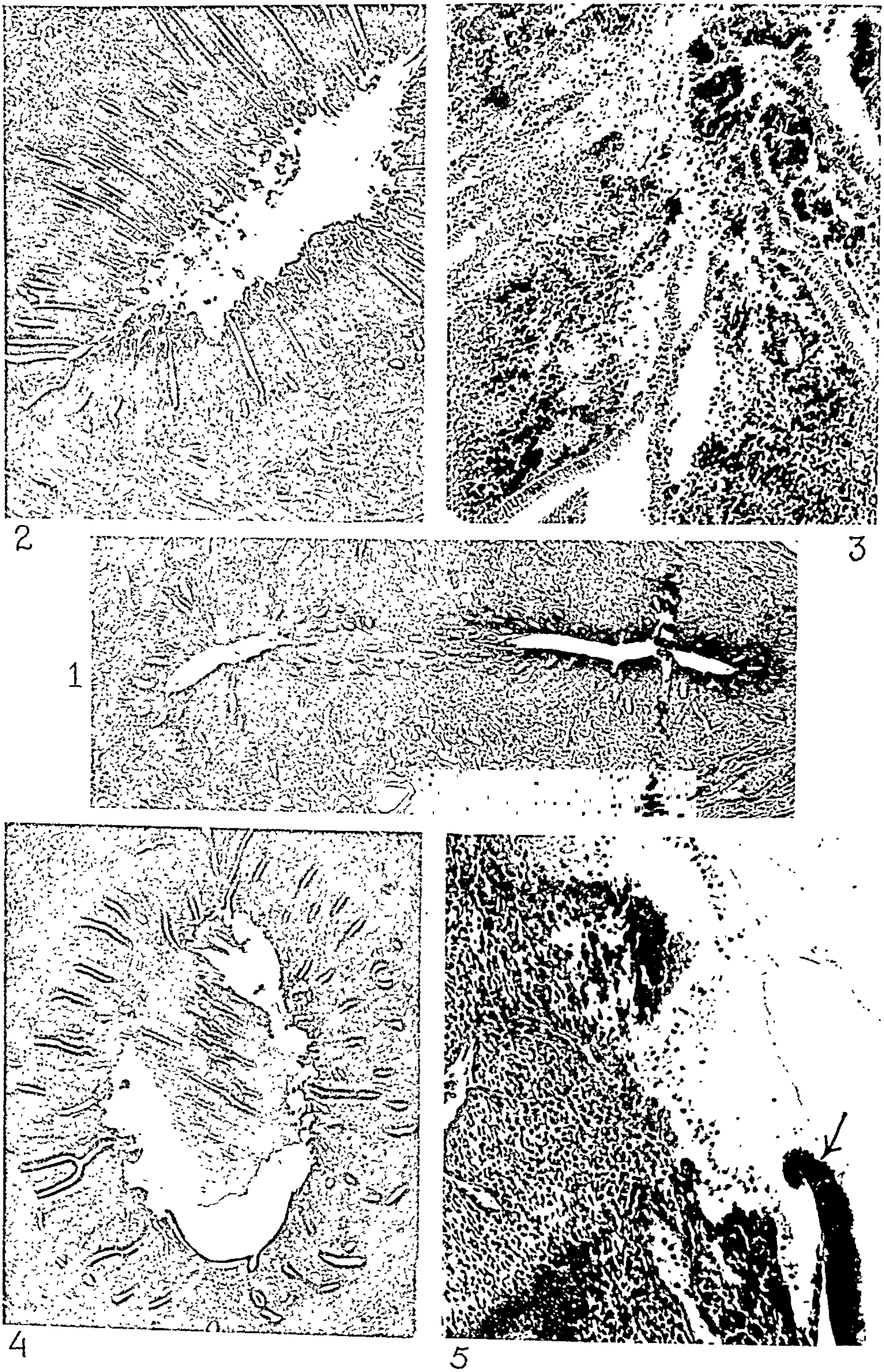
After this experiment this castrated macaque was used for other experiments, being injected with follicular hormone.

On September 17, when the animal weighed 3825 grams, the injection of the hypophyseal extract was begun again, amounting to 40 cc. in 9 days. No bleeding was seen either macroscopically or microscopically. Reddening and swelling were not detected during this period at all.

Macaque 93, weight 2470 grams, immature. After the castration, bleeding was seen because the extract of the hypophysis had been administered previously to produce it. Two weeks after the cessation of the external bleeding the injection of the hypophyseal extract was begun, amounting to 31 cc. in 8 days. Neither macroscopical nor microscopical bleeding was seen during or after the treatment, and no reddening or swelling was detected.

Macaque 49, weight 3825 grams, mature. This mature macaque had been menstruating regularly and was in good health. The last menstruation was observed for 5 days beginning August 24, 1931. On September 4, both ovaries were removed. Bleeding was detected again on September 9 and lasted for 5 days and microscopically for 2 days more. This must have been the result of the oöphorectomy, as noted by E. Allen (1926). On September 23 injection of the hypophyseal extract was begun, amounting to 40 cc. in 7 days. No bleeding was observed during or after the treatment. Neither swelling nor distinct increase of reddening was seen.

These experiments show, therefore, that injection of hypophyseal extracts into castrated monkeys caused no bleeding (6 cases using 4 macaques), whereas the same extract when injected into immature non-castrated monkeys did cause bleeding. It is logical to suppose then that this bleeding is dependent upon the presence of the ovary, and that the uterine changes follow the induction by the hypophyseal extract of some alteration in the ovary. Moreover, it must be added here that when bleeding was not produced by injections (the third experiment on no.



Figs. 1–5

70, non-castrated), swelling of the vulva was very slight when compared with that seen in the other cases.

THE UTERUS. The uterus was observed in several cases by exploratory laparotomy and in some instances was studied microscopically following hysterectomy.

Macaque 82. On the second day of the experimental bleeding a laparotomy was performed. The uterus was not notably larger than in normal immature monkeys of the same age.

Macaque 70. After the second series of injections, i.e., 2 weeks after the onset of the first experimental bleeding and 5 days before the onset of the second experimental bleeding, the uterus (observed by laparotomy) was as large as a mature uterus.

Macaque 69. Two weeks after the onset of the first bleeding and a week before the second bleeding, the uterus was as large as that of a mature animal. On the second day of the third experimental bleeding the uterus was removed and much blood was found in the cavity. Microscopical sections showed bleeding and desquamation from an interval stage of the endometrium (figs. 2, 3), just the same as in cases of normal spontaneous menstruation (without ovulation) described by Corner and E. Allen. It was found that the endometrium was thicker than normal, there was ragged mucosa with sloughing of superficial glands, and much blood. The glands were straight and not dilated, the gland epithelium was high, epithelial mitoses were absent and there were many lakes of extravasated blood in the subepithelial tissue.

Macaque 72. On the second day of bleeding the uterus was removed and found to be enlarged and hyperemic and to contain much blood in the cavity. The histological examination showed almost the same findings as in no. 69 (figs. 4, 5). Moreover many subepithelial capillaries were seen to be much dilated and connected with lakes of extravasated blood.

THE OVARY. *Macaque 70.* After the series of injections 5 days before the experimental bleeding, the exploratory operation showed several small follicles at the surface of each ovary but no recent or old corpora lutea were seen.

Macaque 82. On the second day of the experimental bleeding the exploratory operation showed that the ovaries were rather small and had a few small follicles but no corpora lutea were visible.

Macaque 72. On the second day of the experimental bleeding, the ovaries were

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Fig. 1. Section of uterus of a normal immature macaque. Photograph, × 15. Hematoxylin and eosin. Endometrium is much thinner than in the pictures which follow.

Fig. 2. Section of endometrium, macaque 69. Photograph, × 15. Section shows desquamation of the epithelium and superficial portions of the glands. Blood in the cavity. Glands of interval type.

Fig. 3. Higher magnification (× 100) of the endometrium shown in figure 2. Ragged mucosa with sloughing of superficial glands. Blood in cavity and many lakes of extravasated blood in subepithelial zone.

Fig. 4. Section of uterus, macaque 72. × 15. Blood in cavity, desquamation of the epithelium. Glands of interval type.

Fig. 5. Higher magnification (× 100) of the endometrium shown in figure 4. Epithelium has disappeared or is being desquamated (see arrow). Dilated capillaries full of blood appear to be connected with lakes of blood.

removed with the uterus and examined microscopically. Neither large follicle near ovulation nor corpus luteum was found.

Macaque 69. In the interval between the two experimental bleedings the ovaries were found by exploratory laparotomy to be as large as those found in fully mature animals. Many small follicles were present but no large follicles nor corpora lutea were observed. On the second day of the third experimental bleeding the ovaries were removed. Microscopic sections showed many medium and small-sized follicles

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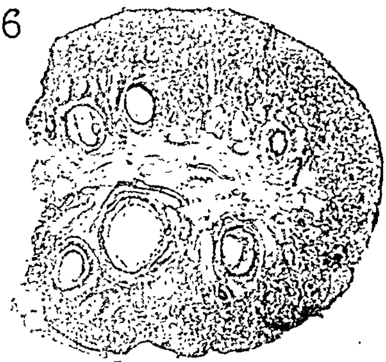

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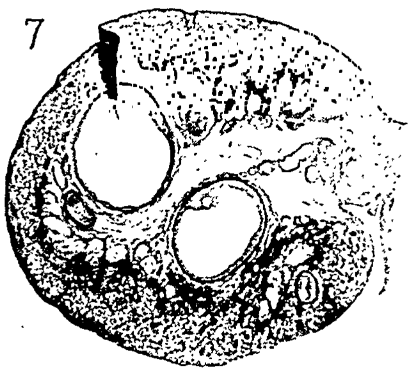

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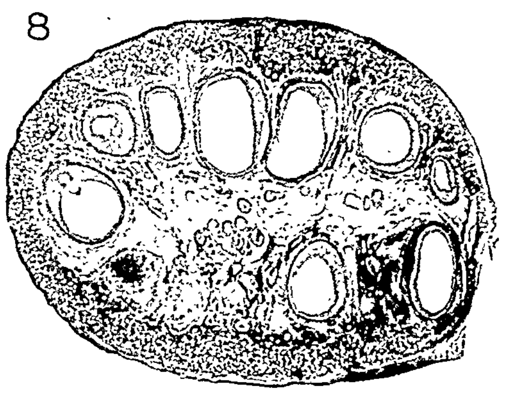

Fig. 6. Section of the right ovary, macaque 93, just before the second injection of the smallest amount of the hypophyseal extract sufficient to cause bleeding. Photograph, × 10.

Fig. 7. Section of the left ovary, macaque 93, just after the above mentioned injection (15 cc.). Follicles are distinctly larger than follicles of the right ovary (fig. 6), but otherwise not much different from the right. Photograph, × 10.

Fig. 8. Section of the right ovary, macaque 29, after three series of injection of the hypophyseal extract. Follicles are more in number and distinctly larger than in the ovary of another animal of the same size (fig. 6). Much vascularisation is to be seen. Neither corpus luteum nor large follicle near ovulation. Photograph, × 10.

in which liquor folliculi had been elaborated. There were 31 medium and 27 small follicles in the right and 26 medium and 39 small follicles in the left. They showed neither large follicles nor corpora lutea, consequently ovulation had not occurred. But when the ovaries of this animal are compared with those of the immature monkey of about the same age (fig. 6, 8) it is clearly seen that considerable follicular development had occurred.

A series of experiments, to be added here, will sustain the idea that the bleeding occurs when the stimulating effect of follicular hormone upon the endometrium is decreased after previously causing some changes in it.

In the first experiment of this series, it was attempted to elicit bleeding by injection of the follicular hormone into a castrated immature macaque.

Macaque 68, 3450 grams. This castrated immature macaque was first used for a control experiment, being injected with the extract of the hypophysis, but it was found impossible to produce bleeding in this way (see p. 11). Next, she was injected with 245 R. U. of follicular hormone (amniotin) over a period of 9 days. Reddening began on the fourth day (after the injection of 45 R. U.), and swelling began on the sixth day (after the administration of 75 R. U.). Nine days after the last injection the lavage had a reddish tinge, and on the following day much external bleeding began, which lasted for 6 days. Microscopical bleeding was seen for one day before and for 4 days after this period.

Seven weeks later 60 R. U. of the follicular hormone were given over a period of 5 days. Reddening began on the third day and swelling was seen on the fifth day. Thirteen days after the last injection the lavage showed a red tinge and contained many red cells. The next day external bleeding began and lasted for 4 days. Very few red cells were seen microscopically for 6 days before that and microscopical bleeding lasted 5 days after the external bleeding.

In this experiment, just as in those of E. Allen and Morrell, external bleeding was produced in a castrated immature macaque by injecting follicular hormone. The bleeding occurred several days after the cessation of the administration of the hormone. The doses used caused reddening of the sexual skin and swelling of the vulva and probably stimulated the endometrium too.

The fact that uterine bleeding could be produced by treatment with follicular hormone in this castrated immature animal demonstrates that failure to obtain the same result with hypophysis extracts in previous experiments on the same animal was not due to insensitiveness of the uterus.

In the next experiment an immature animal was given a small amount of hypophysis extract, insufficient to induce bleeding, but sufficient to cause some stimulation of the ovaries as evidenced by reddening of the sexual skin. The animal was then spayed.

Macaque 68, 3390 grams, immature. Before the castration, 13 cc. of hypophysis extract had been injected over a period of 4 days. This amount is thought to be insufficient to cause the external bleeding from the uterus (see the experiment on

to bleed three times at rather short intervals by the administration of the hypophysis extract, showed many follicles larger than those of a normal immature macaque.

Since it is clear that the menstruation-like bleeding is closely correlated with the function of the ovary, much histological difference between those ovaries might have been expected; but the above mentioned data show rather that the small amount of follicular hormone sufficient to cause the bleeding and some other ovarian symptoms can be secreted from an ovary without any marked histological changes.

The mechanism of this experimental menstruation-like bleeding, produced by the injection of an extract of the hypophysis, remains to be discussed. It is unnecessary from these experiments to postulate a separate anterior lobe hormone as the direct cause of menstruation and much more logical to explain this menstruation-like bleeding as the result of stimulation of the ovaries by the anterior lobe hormone because: 1. The spayed monkeys did not bleed. 2. The menstruation-like bleeding did not occur during the injection of the extract but only several days after the treatment. 3. The bleeding was always preceded by the secondary sex characteristics of reddening and swelling of the sexual skin, and the uterus showed very clearly enlargement, congestion, and increased thickness of endometrium. All of these signs are known to be produced by the follicular hormone. 4. Though there were no large follicles near ovulation or corpora lutea in the ovaries at the time of the menstruation-like bleeding, some development of the ovaries was recognizable. 5. As shown by others and confirmed in these experiments, similar bleeding often occurs a few days after oöphorectomy, and is produced in the spayed monkey following the administration of follicular hormone. 6. The bleeding which invariably follows injection of the hypophysis extracts may be postponed by administration of follicular hormone.

On the basis of these experiments, it is tentatively assumed that the menstruation-like bleeding of the endometrium occurs after a certain physiological state of the endometrium caused by the follicular hormone is altered when the hormone is removed or reduced in amount. The effect of the hypophysis extract is, under this interpretation, simply to stimulate production of the follicular hormone, which is then reduced in amount after cessation of the hypophyseal injections. It seems to me to be more difficult to explain this bleeding as a result of the action of a special substance from the hypophysis.

SUMMARY

1. Menstruation-like bleeding from the uterus was caused by the injection into immature monkeys of an extract of the hypophysis (11 positive cases in 12). Intervals between the last injection and the external bleed-

ing were from 4 to 9 days, and the duration of the external bleeding was from 5 to 7 days.

2. This menstruation-like bleeding was produced 2 or 3 times at short intervals.

3. Castrated monkeys did not bleed microscopically or macroscopically during or after the injection of the anterior lobe extract

4. The ovaries of the monkeys in which bleeding was produced by the treatment showed no ripe follicles or corpora lutea but there was some difference in the ovaries, as shown by experiments in which the follicles were larger in the ovaries removed after the injections than in the control ovaries. This experimental menstruation-like bleeding was similar to that found in normal monkeys where regular menstruation may occur without rupture of a follicle or presence of a corpus luteum.

5. The uteri from the positive cases showed bleeding from an interval stage of the endometrium.

6. A castrated immature monkey, which did not bleed following injections of hypophysis extract, showed menstruation-like bleeding subsequently after a course of injections with follicular hormone.

7. An immature monkey treated with a sub-effective dose of hypophysis extract and then spayed, showed bleeding after removal of the ovaries.

8. Bleeding which normally follows treatment with hypophysis extract was postponed by injections of follicular hormone.

9. It is assumed from the above experiments that the experimental menstruation-like bleeding results from an alteration of the endometrium which occurs when the effect of follicular hormone is removed.

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A QUANTITATIVE STUDY OF PLACENTAL TRANSMISSION AND THE PERMEABILITY OF FETAL MEMBRANES AT VARIOUS STAGES OF PREGNANCY

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In recent observations on the placental transmission of certain hormones following injection of them into the fetus, Snyder and Hoskins (1928 a, b) obtained no evidence of changes in the maternal organism that could be attributed to escape across the placental boundary of the injected substances, namely, insulin, adrenalin, pituitrin, and parathyroid. Accordingly, phenolsulphonphthalein was added to the substances to be injected into the fetus, in order to get evidence that the injected material had reached the fetal circulation, which would be shown by recovery of phenolsulphonphthalein from the maternal urine.

It became evident at once in these injections of phenolsulphonphthalein together with endocrine extracts that the escape of phenolsulphonphthalein across the placental boundary and appearance in the maternal urine occurred at a rapid rate and in considerable amount. The maternal urine was deeply colored with the dye in less than half an hour. This confirmed, in the rabbit, the previous observations of Wislocki (1921), who showed that phenolsulphonphthalein injected into guinea pig fetuses in utero could be recovered in the maternal urine forty minutes following injection.

In view of the well-known usefulness of phenolsulphonphthalein in obtaining quantitative data on kidney function in the human by merely comparing the amount of dye recovered in the urine in a given time with the amount injected, it seemed likely that quantitative measurement of the excretory processes of the fetus might be obtained in a similar manner, by determination of the output of phenolsulphonphthalein in the maternal urine, following the injection of the dye into fetuses in utero.

The selection of animals at various stages of pregnancy permitted detection of any change in amount of excretion as gestation approaches term.

In a second series of animals in which phenolsulphonphthalein was injected into the gestation sac, the attempt was made to measure the permeability of the chorio-amniotic membranes beyond the placental site

and to gain an estimate of the efficiency of the barrier between the amniotic fluid and maternal blood stream at various stages of pregnancy.

METHOD AND MATERIAL. The present experiments may be considered in three main groups, as summarized in tables 1, 2, and 3.

In the first group, the output of phenolsulphonphthalein was measured under uniform conditions of technique. Only the age of the embryos was varied in this series of observations.

In the second group, uniform conditions were similarly maintained except that the amount of phenolsulphonphthalein injected per fetus was varied; and in one experiment the number of fetuses injected was increased.

TABLE 1

Output of phenolsulphonphthalein in maternal urine following injection of fetuses in utero with 3 mgm. (0.5 cc.) per fetus

Stage of pregnancy	Rabbit number	Weight of fetus	Number of fetuses injected	Phthalein injected per fetus	Phthalein injected per gram of fetus	Output of phenolsulphonphthalein: Period 1, 0–3½ hour (per cent of total, 6 mgm.)	Period 2, 3½–6th hour (per cent of total, 6 mgm.)	Period 3, 6th–12th hour (per cent of total, 6 mgm.): 1st 3 hours	Period 3: 2nd 3 hours	Total in 6 hours	Total in 6 hours	Total in 6 hours (mgm. per gram fetal body weight)	Control (output of maternal animal 2 days following fetal injection): Phthalein injected	Control: Output of phthalein in 2½ hours
days		grams		mgm.	mgm.					per cent	mgm.		mgm.	per cent
21½	9	8	2	3	0.38	19	13			32	1.9	0.12	6	83
22	19	8	2	3	0.38	17	15	14	14	32	1.9	0.12	6	84
22½	11	6	2	3	0.50	19	14			33	2.0	0.16	6	80
23	8	11	2	3	0.27	17	18			34	2.0	0.09	6	
24	13	17	2	3	0.18	11	11			22	1.3	0.04	6	93
26	3	20	2	3	0.15	9	6			15	0.9	0.02	6	78
28	4	27	2	3	0.11	8	6			14	0.8	0.01	6	
29	23	32	2	3	0.07	10	9	3	3	19	1.1	0.02	6	82
29½	6	(35)	2	3	0.09	8	5			13	0.8	0.01	6	95

In the third group, observations were made under conditions identical with the first group except that phthalein was injected into the gestation sac instead of into the embryo.

The procedure in a typical experiment was as follows. The animal was fastened to the operating table on its back. A soft rubber tube was passed into the stomach through which amytal was given slowly, using 0.1 gram per kilo body-weight made up to a total volume of 200 cc. The large amount of water promoted adequate diuresis. The abdomen and genital region of the animal were shaved. Collection of urine by catheter is usually diffi-

cult in the female rabbit owing to the situation of the urethral orifice internally at a distance of about 10 cm. from the external opening of the

TABLE 2

Output of phenolsulphonphthalein in maternal urine following injection of fetuses in utero with various amounts of dye

Stage of pregnancy	Rabbit number	Weight of fetus	Number of fetuses injected	Phthalein injected per fetus	Phthalein injected per gram of fetus	Output of phenolsulphonphthalein: Period 1, 0–3½ hours (per cent of total injected)	Period 2, 3½–6th hour (per cent of total injected)	Period 3, 6th–12th hour (per cent of total injected)	Total in 6 hours		Total in 6 hours (mgm. per gram of fetus)	Control (output of maternal animal 2 days following fetal injection): Phthalein injected	Output of phthalein in 2½ hours
days		grams		mgm.	mgm.				per cent	mgm.		mgm.	per cent
22	16	7	4	1.5	0.21	8	13		21	1.3	0.05	6	85
29	14	35	2	18.0	0.51	10	9		19	6.8	0.09	6	81
29	15	45	2	18.0	0.40	12	8		20	7.2	0.08	6	85

TABLE 3

Output of phenolsulphonphthalein in the maternal urine following injection of the gestation sac with 3 mgm. (½ cc.) of dye per fetus

Stage of pregnancy	Rabbit number	Weight of fetus	Volume of fluid of gestation sac	Surface area of fetal membranes (est.)	Phthalein injected per fetus	Number of sacs injected	Output of phenolsulphonphthalein: Period 1, 0–3½ hours (per cent of total, 6 mgm.)	Period 2, 3½–6th hour (per cent of total, 6 mgm.)	Period 3, 6th–12th hour (per cent of total, 6 mgm.)	Total in 6 hours		Control (output of maternal animal 2 days following fetal injection): Phthalein injected	Output of phthalein in 2½ hours
days		grams	cc.	sq. cm.	mgm.					per cent	mgm.	mgm.	per cent
21	10	7	3	20	3	2	4	8		12	0.7	6	80
21	270	7	3	20	3	2	4	7	27	11	0.7		
25	22	15	5	35	3	2	11	6	8	17	1.0	6	87
26	21	14	5	35	3	2	11	16	11	27	1.6	6	72
29	20	47	1	60	3	2	42	12	12	54	3.2	6	78
30	17	30	2	50	3	2	43	5		48	2.9	6	
30	18	35	2	40	3	2	40	11		51	3.1	6	90

urogenital tract. Accordingly a funnel emptying into a flask was fitted tightly against the perineum. A braided silk thread attached to the skin

of the vulva was carried through the funnel and then over the neck of the flask to the table where it was held under tension.

Within an hour after administration of amytal, when the animal was deeply anesthetized, a lower midline incision was made under aseptic precautions and the uterus was exposed. The fetus was palpated and grasped below the costal margin so that a fine gauge needle could be inserted in the region of the dorsal musculature, passing through the thin uterine wall and enveloping membranes. The barrel of the syringe was then fitted into the needle, thus limiting the possibility of leakage into the maternal tissues during insertion of the needle into the embryo. A cotton sponge also surrounded the base of the needle near the junction with the syringe in order to detect and prevent escape of phthalein at this point. Care was taken to handle the uterus as little as possible especially near the placental site, and to avoid disturbance of the circulation such as is inevitable if the uterus is delivered through the abdominal wound. After the twenty-ninth day of pregnancy, however, premature expulsion of the fetuses becomes increasingly likely as term is approached, despite the above precautions. The abdomen was closed rapidly in two layers with purse string silk suture.

Under the influence of amytal the animal remained quietly on its back throughout the period of observation which was usually six hours, although occasionally as long as twelve hours.

Phenolsulphonphthalein is obtained in 1 cc. ampules which, as made up by Hynson, Westcott and Dunning for clinical use in test of renal function, contain 6 mgm. phenolsulphonphthalein per cubic centimeter of solution. Each fetus received a dose of 3 mgm. or 0.5 cc. phthalein solution, and two fetuses were injected with a tuberculin syringe graduated in 0.01 cc.

The urine collected during $3\frac{2}{3}$ hours following the injection of the embryos was isolated from the specimen obtained during the subsequent $2\frac{1}{3}$ hour period. When the experiment was continued over a twelve-hour period, the urine of the final six hours was isolated. At the conclusion of each period of the experiment the abdomen was opened again and the bladder filled with saline by direct injection through a fine needle, and then emptied in order to wash out thoroughly any remaining dye from the urogenital tract. About 25 cc. of saline were used in this way at each washing.

At the termination of the experiment the uterus was opened and the fetuses examined to see whether they were alive, as determined by beating of the heart. No experiments were included in which the death of a fetus occurred. The injected fetuses were then weighed and the average weight per fetus was recorded in the table. After emptying the uterus and removing the placentas, which could be accomplished with little loss of blood, the abdominal incision was sutured.

The quantity of phthalein recovered in the urine was then determined

colorimetrically. A convenient standard was made by diluting 0.5 cc. or 3 mgm. of the dye to a volume of one liter with distilled water and adding a few cubic centimeters of 25 per cent KOH until the color was fully developed. The urine containing an unknown amount of dye was then similarly made alkaline until the color no longer deepened and was diluted with water until the color matched that of the standard. Having determined the volume of the unknown urine specimen at the match point with the standard, the amount of phthalein was readily calculated. Error in colorimetric comparison arising from the pigments of the urine was eliminated almost entirely by this process of dilution as well as by the watery nature of the urine following diuresis resulting from the water intake of 200 cc.

About two days later the animal was injected intramuscularly with 6 mgm. of phthalein under amytal anesthesia as previously, and the output of phthalein in the urine during 2½ hours was determined. Thus the ability of the maternal animal to excrete phthalein in the urine was controlled by direct observation.

The stage of pregnancy could be accurately estimated since the animals were mated in the laboratory. Ovulation regularly occurs in this species about ten hours following copulation. The gestation period is about thirty-two days. The rabbits were reared in the laboratory on a liberal diet of oats, alfalfa, cabbage, salt and water.

Observations. *Group I.* In the first series of animals, as summarized in table 1, it is evident that during the last third of pregnancy there was a striking change in the output of phenolsulphonphthalein in the maternal urine following injection of fetuses in utero. When two fetuses of a litter were injected with 3 mgm. or 0.5 cc. phthalein per fetus, it was found that at the twenty-second day of pregnancy about 32 per cent of the phthalein was recovered within a period of six hours following injection, while near term, at twenty-nine days, only about 13 per cent was obtained. At an intermediate stage, in fetuses injected on the twenty-fourth day of pregnancy, it was found that the amount of phthalein recovered was likewise intermediate between the two extremes, being about 22 per cent of the total amount injected. The amount of phthalein which escapes across the placental boundary into the maternal blood stream is obviously much greater early in pregnancy than near term.

In control observations on the excretion of phthalein from the maternal organism following intramuscular injection of 6 mgm., at least 80 per cent of the dye was recovered in the urine within 2½ hours, indicating that the maternal kidney function was normal.

Additional details of the experiments, as recorded in table 1, are illustrated in figure 1. It is pointed out that the rate of excretion of phthalein tends to be more rapid early in pregnancy in both the first period of 3⅔

hours after injection and the second period of $2\frac{1}{3}$ hours. Thus in the experiment with no. 19, which was injected on the twenty-second day of pregnancy, the output in the first period was 17 per cent of the amount of phthalein injected, and in the second period was 15 per cent. On the other hand, in no. 6, which was injected on the twenty-ninth day, the output in the first period was about 8 per cent, while in the second period it was only 5 per cent.

In one animal, no. 19, observed on the twenty-second day of pregnancy, in which measurement of the phthalein output was extended over an additional six-hour period, making a total of twelve hours, about 60 per cent of

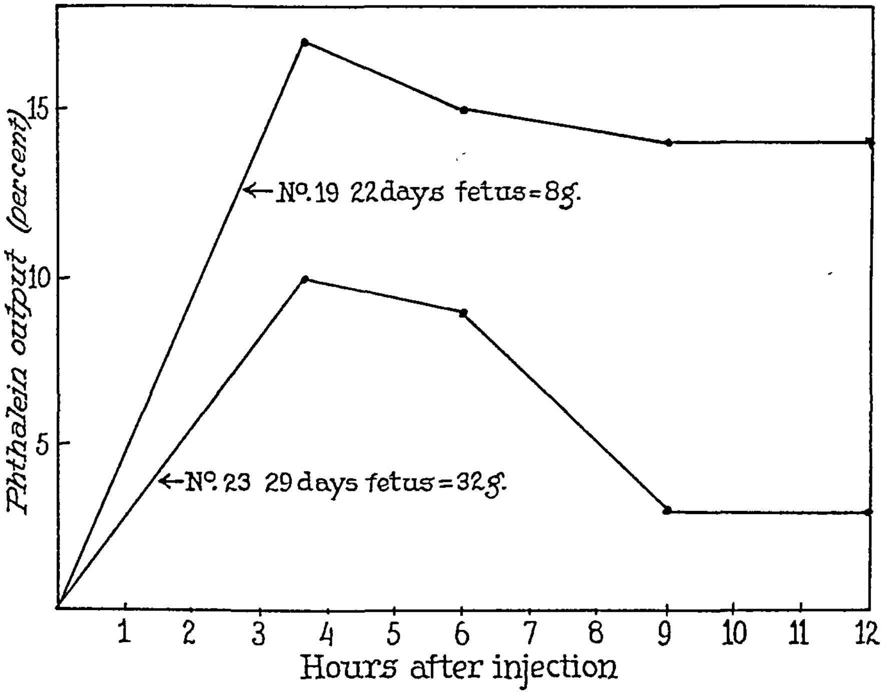

Fig. 1. Output of phenolsulphonphthalein in the maternal urine following injection of the foetuses at the 22d and 29th day of pregnancy respectively.

the dye was recovered. Since the output during the final six hours equalled 28 per cent of the amount injected, or about 14 per cent in each of two periods of three hours, there is evidence that the excretion of phthalein is maintained with little diminution during this period as indicated in figure 1.

In addition to this correlation of the response of the excretory mechanism of the fetal organism following injection of a constant amount of phthalein per fetus, with the age of the embryos, it is interesting also to take into account the change in weight of the fetuses, table 1. Since the weight of a fetus at twenty-two days averages about 8 grams while in a litter observed at twenty-nine days the weight is 35 grams, it is evident that when 3 mgm.

of phthalein are injected into each fetus, the dose per gram body-weight of the embryo is about 0.38 mgm. in the smaller fetus as compared with 0.09 mgm. in the larger, or four times as great. It is found that the smaller fetus excretes more than twice as much phthalein in six hours as the larger, the output being about 1 mgm. from the smaller compared with less than ½ mgm. from the larger embryo. If the output per gram body-weight is considered, the difference between the smaller and larger fetus is striking. The fetus weighing 8 grams eliminates twelve times as much phthalein per gram body-weight as the fetus weighing 35 grams, or about 0.12 mgm. phthalein per gram body-weight in the former compared with 0.01 mgm. in the latter, during the period of six hours.

Group II. The next question, namely, the influence of increasing or decreasing the amount of phthalein injected, is considered in the experiments summarized in table 2.

In experiments with no. 14 and no. 15 the amount of phthalein injected into fetuses on the twenty-ninth day of pregnancy was increased in an attempt to equal the dosage per gram fetal body-weight which was injected into the smaller embryos of the twenty-second day of pregnancy. Thus in no. 15 with fetuses weighing 45 grams the amount of phthalein was increased six-fold, each fetus receiving 18 mgm. or 3 cc., thus giving a dosage of 0.40 mgm. phthalein per gram fetal body-weight. This closely parallels the amount given per gram body-weight in no. 19 with fetuses weighing 8 grams. With this increased dosage, the output of no. 15 was found to be about 20 per cent of the amount of phthalein injected, in contrast to 32 per cent in no. 19 with 8 gram embryos which received the same dosage of phthalein per gram body-weight. If the output per gram fetus body-weight is considered, it is found likewise that the output of the larger fetus is less than the smaller, being 0.08 mgm. per gram body-weight in the 45 gram fetus in contrast to 0.12 mgm. in the 8 gram fetus.

In the experiment with no. 16, having embryos of the twenty-second day of pregnancy weighing 7 grams, the injection of 6 mgm. or 1 cc. phthalein was divided among four embryos, each receiving 1.5 mgm. or ¼ cc. As a result the total output of phthalein was decreased to 21 per cent in six hours instead of about 32 per cent output which was observed when only two fetuses were injected with 6 mgm. of phthalein, each receiving only 3 mgm. Likewise the excretion per gram fetal body-weight was lowered to less than half the amount found after two fetuses were injected, being about 0.05 mgm. per gram body-weight when four embryos were injected, in contrast to 0.12 mgm. when two fetuses were injected. It is seen also that a greater amount of phthalein was eliminated in the second period after injection in no. 16 than in the first $3\frac{2}{3}$ hours. About 8 per cent of the amount injected was recovered in the first period while 13 per cent was excreted in the second part of the total six hours.

It is interesting to note that at the end of the experiments, the bladders of the fetuses that were injected with phthalein contained the dye, although not in sufficient quantity to be considered in the calculations of output. By sampling the bladder urine with a needle and syringe it was found easy to identify among the litter the fetuses that had been injected with phthalein.

Group III. In the third group of experiments as outlined in table 3, phthalein was injected into the waters surrounding the embryo instead of

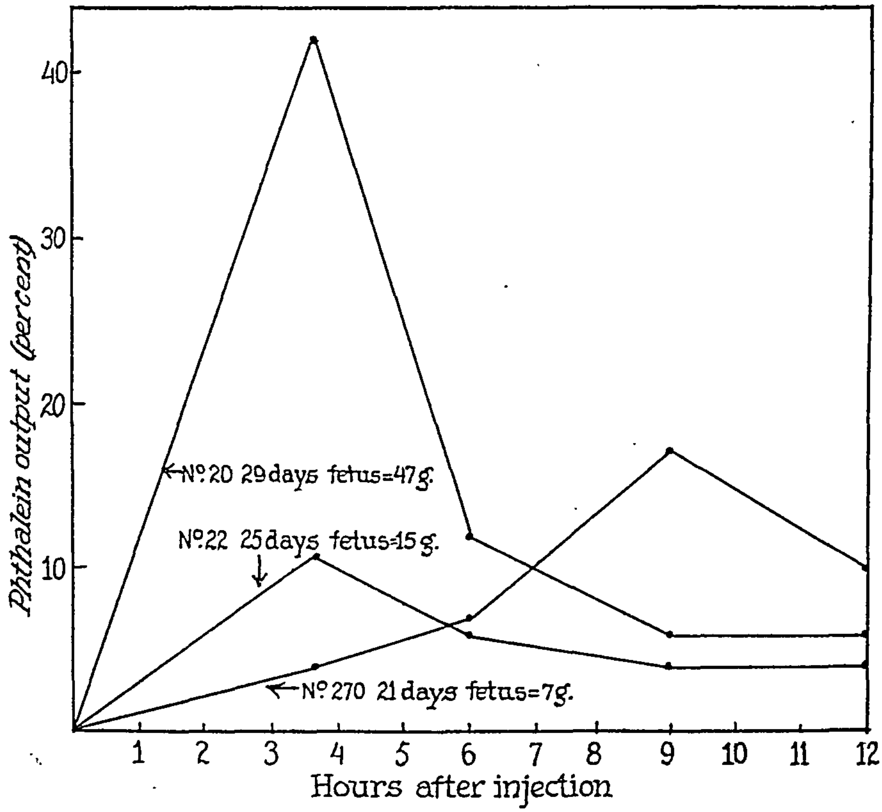

Fig. 2. Output of phenolsulphonphthalein in maternal urine following injection of the amniotic sac at the 21st, 25th, and 29th day of pregnancy.

into the fetal body. In each experiment the amniotic sac was injected in two fetuses, using 3 mgm. or $\frac{1}{2}$ cc. phthalein in each instance, or a total injection of 6 mgm.

Observations under these uniform conditions gave striking evidence of changes in the output of phthalein from the gestation sac at various stages of pregnancy. In an animal, no. 10, observed on the twenty-first day of pregnancy, about 12 per cent of the phthalein escaped within six hours, while in an animal near term, no. 18, the thirtieth day of pregnancy, about 51 per cent of the dye was recovered, or four times as much. At an inter-

mediate stage, as in no. 22 (fig. 2) observed on the twenty-fifth day of pregnancy, only 17 per cent of the phthalein escaped into the maternal circulation in six hours. Indeed in this animal observation was extended over a period of twelve hours during which the total output was about 25 per cent, which was less than the amount excreted in the first period of $3\frac{2}{3}$ hours at the twenty-ninth day of pregnancy in no. 20.

If the output of phthalein is considered during the first period of $3\frac{2}{3}$ hours following injection, it is found that the excretion near term is about ten times greater than at the beginning of the last third of gestation. In an animal, no. 10, observed on the twenty-first day of pregnancy the output was 4 per cent in the first $3\frac{2}{3}$ hours while in no. 18, studied on the thirtieth day of pregnancy, about 40 per cent of the dye was recovered during this same interval. In the second period of collection, the excretion fell off markedly in no. 18, amounting to 11 per cent, while in no. 10 there was an increase to 8 per cent. During the entire six-hour period, therefore, the output is about four times as great near term as at the beginning of the last third of pregnancy. If the experiment is extended over a twelve-hour period, it is found that the output is maintained at a fairly low level during the final six hours, figure 2.

In connection with these findings there comes into consideration the change in surface area of the fetal membranes and the variation in the amount of fluid contained in the gestation sac with consequent change in dilution of the injected dye at various stages of pregnancy. In table 3 the volume of fluid is given as in the tabulation of Bruno Wolff (1909), which we also had opportunity to check in the course of the experiments. The surface area is calculated from the volume of the sac which is taken roughly to approximate the sum of the weight of the fetus plus the surrounding fluid. It is evident that as term is approached, the surface area of the fetal membranes is increased and the volume of fluid is diminished in amount so that there is less dilution of the injected dye at the surface of the limiting membrane. Both changes are in the direction of promoting increased elimination of phthalein, which of course supports the experimental findings. The magnitude of these physical changes, however, seems hardly adequate alone to account for the marked differences in output at various stages of pregnancy.

Discussion. In the foregoing observations, quantitative data have been obtained regarding the excretory processes of the fetus in utero. Although in the past countless substances have been injected into fetuses and into the amniotic sac in various species, this study is the first, as far as we know, that is based upon quantitative measurement of the excretory function.

The two anatomical channels along which products of fetal metabolism can conceivably reach the maternal blood stream have been studied in

separate series of animals. By aid of a dye, phenolsulphonphthalein, which is well known through its use in the study of human excretory function, and which is not toxic for the fetus, it has been possible to obtain a record, hour by hour, of the amount of the foreign substance which escapes by the two routes, that is, either across the placental boundary, or directly through the fetal membranes over an area less specialized than the placental region.

By selection of fetuses at various stages during the last third of pregnancy, during which period the fetal body-weight undergoes a five-fold increase, it has been possible to discover changes in excretion which might easily remain inconspicuous under less favorable conditions of observation.

An attempt has been made to correlate the output of phthalein across the placental boundary with various factors which could be kept under control, such as age of the fetus, weight, number injected, dosage of the dye, and time interval. Similarly in observations on the output directly through the chorio-amniotic membranes, correlation has been considered with regard to such factors as the surface area of the sac and the influence of dilution of the injected dye by fluid of the sac upon the final concentration at the surface of the limiting membrane. However, in explanation of the decrease in placental transmission of phthalein as term is approached and the increase in permeability of the fetal membranes to the dye, it is difficult to escape the conclusion that these changes represent in a certain measure a physiological parallel to the histological evidence (Mossman, 1926) of senility of the placenta and membranes as parturition draws near.

SUMMARY

1. In a study of placental transmission in a series of rabbits observed during the last third of gestation, it was found that following the injection of fetuses in utero with 3 mgm. of phenolsulphonphthalein per fetus there is an output during a six-hour period of more than 30 per cent of the dye from the fetus at the twenty-second day of pregnancy, while less than 15 per cent of the dye is excreted from the fetus at the twenty-ninth day.

2. If allowance is made for a sixfold increase in weight of the embryos near term, and the amount of phenolsulphonphthalein injected per fetus is increased to 18 mgm., there is still an output during a six-hour period of only 20 per cent of the dye from the fetus at the twenty-ninth day.

3. In a study of the permeability of fetal membranes in a series of rabbits observed during the last third of gestation, it was found that following the injection of the gestation sac with 3 mgm. of phenolsulphonphthalein per fetus there is an output during a $3\frac{2}{3}$ hour period of about 4 per cent of the dye from the sac at the twenty-first day of pregnancy, while about 40 per cent of the dye is excreted from the sac at the thirtieth day.

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RHYTHMIC VASCULAR UTERINE CHANGES[1]

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Although a great deal of work has been done on the changes in the uterus during the sex cycle in mammals, very few observations have been made on its rhythmic vascular changes. As reported in 1929, the color of the uterus of the guinea pig undergoes rhythmic variations that make the mucous membrane appear to blush and blanch every 15 to 20 seconds. These vascular changes are influenced by the time of day; being slowest and least intense in the early morning, increasing both in speed and intensity in the forenoon, decreasing again about noon and reaching their height of speed and intensity late in the evening. They are also influenced by the stage of the oestrous cycle, slowing during the pro-oestrum until they disappear completely for four or five hours and then reappearing before the end of the period of heat. The color changes are caused by variations in the amount of blood in the capillaries and arterioles due to rhythmic contractions of the latter. They appear to be specific for uterine tissue. Similar vascular changes were not observed in other tissues *in situ* nor in transplants onto the iris, or pancreas, islands of Langerhans, ductus deferens, heart muscle or liver. That vascular changes like those studied in the transplants also occurred in the uterus *in situ* was proven by observing them in anesthetized guinea pigs *in situ* and in unanesthetized guinea pigs through a uterine speculum.

Since these vascular changes are greatly affected by anesthesia and trauma to the uterus and surrounding tissues, a celluloid window 50 mm. square was placed in the lateral abdominal wall and the mucosa of an opened uterine horn observed through the window. By this method, the endometrium can be observed *in situ* without concomitant anesthesia or trauma and the vascular changes have the same rhythm as large endometrial transplants in the anterior chamber of the eye. The vascular changes observed through the window are modified by the time of day and the stage of the oestrous cycle, just as in the transplants. The only disadvantage of this method is that the inside of the window becomes covered with connective tissue in about six weeks. Since the vascular changes can

[1] A considerable amount of the material presented in this study has been summarized in an abstract (Markee, 1929).

be satisfactorily observed for at least 23 months in iridial transplants most of the observations on the vascular changes were made on iridial transplants and the other three methods were used only as checks on our results.

SPECIFICITY OF THE RHYTHMIC VASCULAR CHANGES FOR UTERINE TISSUE. As a further check on the specificity of the vascular changes for uterine tissue observations were made on other tissues in lightly anesthetized animals and more transplants of pancreas, islands of Langerhans, heart muscle and liver were made to the anterior chamber of the eye. Adrenal, thyroid and the mucosa of the bladder also were transplanted onto the iris and rather extensive observations were made on gastric fistulae and Pavlov pouches. The stomach resembles the uterus in musculature and mucosa and if these vascular changes were general in character, the mucous membrane of the stomach would seem a good place to observe them. Since no vascular changes were found that resemble those in the uterus, we feel justified in concluding that they are specific for uterine tissue.

RELATION OF THE GONADS TO THE VASCULAR CHANGES. A. *The ovary.* A further indication of the specificity of this phenomenon lies in the fact that it does not appear until after puberty. As was stated (Markee, 1929) it was not observed in immature guinea pigs. Therefore uterine autotransplants were made onto the iris of 12 immature animals to discover whether rhythmic vascular changes would appear at sexual maturity. The transplants were made when the animals were 21 days old. In nine of these animals they became vascularized within 12 to 36 hours but there were no rhythmic vascular changes. The animals went into heat for the first time from 92 to 107 days later. In each animal there was a marked growth in the transplant and an increase in its color during the week preceding the first oestrual period. The vascular rhythm was first observed in each of the nine animals during the three days preceding the first oestrual period. When each of these animals was operated on an extra piece of endometrium was removed and transplanted to the eye of her mother. Five of the transplants were successful and the rhythmic color changes appeared in the transplants in all five mothers from 7 to 10 days after transplantation.

The appearance of the vascular rhythm at sexual maturity and within so short a time in transplants from immature daughters to mothers seems to indicate that the uterus must be near or in a mature state before this phenomenon can manifest itself. The appearance of the vascular rhythm in so short a time in the daughter to mother transplants calls to mind the work of Zondek and Ascheim (1926) and P. E. Smith (1926) on the effect of transplants of the oral hypophysis upon the infantile uterus.

Since the color appeared at sexual maturity it seemed advisable to observe the effects of ovariectomy on the vascular rhythm in successful

transplants. Accordingly, 18 guinea pigs were ovariectomized in the middle of their dioestrous periods. For the first 5 days there was no perceptible change in the vascular rhythm. From that time on there was a gradual lengthening of the individual rhythms and a gradual decrease in the vascularity of the whole transplant until by the 45th day the vascular changes had nearly disappeared and the transplant had the appearance of immature endometrium. The time of disappearance of the vascular rhythm varied from 39 to 61 days.

An attempt to determine the effect of ovarian hormone[2] on these vascular changes was suggested both by the disappearance of the vascular phenomenon after ovariectomy and by the continuous blush of oestrus. Injection of the hormone, either subcutaneously or intravenously, into animals with intact ovaries slows the vascular rhythms and within 20 minutes stops them in vasodilatation. The length of time that the blush lasts is roughly proportional to the amount of hormone injected. About $\frac{1}{10}$th of the amount of hormone required to induce the vaginal changes in a spayed guinea pig will induce a period of continuous blush in the uterine transplants.

B. *The testes.* Because of the cumulative evidence that the difference between maleness and femaleness is a matter of a quantitative rather than a qualitative difference, uterine mucosa was transplanted to the eyes of male guinea pigs and rabbits. The transplants in the eyes of males react in all fundamental respects as they do in females. Castration in the male has the same effect on the vascular changes in such transplants, as ovariectomy in the female. After the injection of 20 mouse units of ovarial hormone, twice a day, for a period of a week, the vascular reactions reappear just as they do in the female. Injection of ovarial hormone into males with intact testicles causes a period of vasodilatation just as in the female. Since the same amount of ovarial hormone is required in both the male and female to produce the above changes, it seems that there is no antagonism between the internal secretion of the testicles and the ovarial hormone, at least not in so far as the induction of the period of persistent vasodilatation is concerned. The endometrial transplants in the eyes of males persisted without any alteration in the vascular rhythm for 23 months and microscopic examination did not reveal any degeneration in the mucosa at the end of that time. It therefore seems that a substance is elaborated somewhere in the tissues of noncastrated males that prevents the degeneration of the endometrium and that this substance is lacking in castrated males.

C. *The relation to ovulation.* The temporal relation in the guinea pig between the period of continuous blush and ovulation was studied in a

[2] The preparation used was the ovarial hormone menformon of Laqueuer (1927) obtained from the Marvel Pharmacal Co., New York.

series of 11 animals. One ovary and the corresponding Fallopian tube was removed. If ovulation had occurred the ova were collected from the tube by Corner's method, transferred to a slide and studied under an oil immersion lens. They were measured and the number of polar bodies recorded. If ovulation had not occurred, ovulation was induced by puncturing a folicle and exerting slight pressure on the ovary near the latter. The ovum and the follicular fluid that flowed out of the opening were transferred to a slide and studied. In this way it was found that from 6 to 10 hours elapse between the beginning of the period of continuous blush and ovulation. As seen by table 1 ovulation usually occurs

TABLE 1

The relation of blush to ovulation

GUINEA PIG NUMBER	OVARY	TIME BETWEEN BEGINNING OF BLUSH AND EXAMINATION	TIME BETWEEN END OF BLUSH AND EXAMINATION	OVULATED	STAGE OF MATURATION
		hours	*hours*		
182	Right	6	2	No	Germinal vesicle intact
	Left	8	3	Yes	2nd spindle, 1 polar body
143	Right	7	3	Yes	2nd spindle, 1 polar body
196	Right	6	2	Yes	2nd spindle, 1 polar body
173	Right	5	1	No	Germinal vesicle intact
147	Right	4	0	No	Germinal vesicle intact
	Left	10	5	No	Germinal vesicle intact
157	Right	8	4	Yes	2nd spindle, 1 polar body
	Left	7	2	Yes	2nd spindle, 1 polar body
138	Right	8	4	Yes	2nd spindle, 1 polar body
	Left	6	2	No	Germinal vesicle intact
139	Right	9	4	Yes	2nd spindle, 1 polar body
	Left	7	2	No	Germinal vesicle intact
168	Left	8	4	Yes	2nd spindle, 1 polar body
192	Right	7	3	No	1st spindle
95	Right	7	3	No	No vesicle; spindle not observed

about one hour after the end of the period of blush but in one case it had not even begun five hours after the end of the period of blush. At present all that can be said of this time relation is that the period of continuous blush occurs during the rapid growth of the follicle, at the time of the formation of Robinson's (1918) secondary follicular liquor.

MULTIPLE TRANSPLANTS. As a further test of the specificity of the vascular rhythm, two or more pieces of endometrium were transplanted to the anterior chamber of the same eyes in 253 animals (26 guinea pigs and 227 rabbits). Different transplants in the same eye have rhythms of different lengths. For example, one rabbit had four successful transplants,

two in each eye. Each of the four transplants had an independent rhythm. During a given time (8 minutes) one of the transplants blanched 5 times, second 5, third 7, and the fourth 8 times. Because of this difference in rhythm two transplants will occasionally blanch simultaneously. The frequency of this occurrence, of course, depends on the relative lengths of the rhythms in the different transplants. However, if there is physical continuity between two transplants they have a common rhythm just as the entire uterus *in situ* has a common rhythm.

The rhythm, in the multiple transplants, should be more uniform in length if it were due to a change in the vascular system as a whole. It is, therefore, impossible to explain the vascular cycle in endometrial transplants on the basis of any general rhythmic change in blood pressure or any general rhythmic nervous discharge. Further under these conditions, neither changes in intraocular pressure nor changes in the iris can be offered as the cause of the changes in color.

In order to discover the possible presence of a nervous control of this phenomenon the cervical sympathetic chain was sectioned and stimulated. Although the usual changes were elicited in the pupil and the blood vessels of the ear, the vascular rhythm was not modified by either sectioning or stimulating the cervical sympathetic chain. Further evidence that this phenomenon is not under the direct control of the nervous system is furnished by the fact that the rhythmic vascular changes have been observed and demonstrated as soon as 20 hours after transplantation, and it is highly improbable that a nervous control could have been established within so short a time.

The rhythmic nature of these vascular changes calls to mind the rhythmic contractions of uterine muscle described by Keye (1923). That they are not due to passive compression of the blood vessels by the contraction of the uterine muscle is conclusively proven by transplanting pieces of pure uterine mucosa. However, there probably is some causal connection between the vascular changes and the muscular rhythm, for during oestrus, when the uterus is most congested, the uterine contractions are most powerful. It is impossible to say whether the greater blood supply present at this time causes the increased muscular activity or whether the latter calls for a greater blood supply. It seems more probable that during oestrus both the continuous blush and the characteristic contractions of the uterine muscle are the effects of a modification of general bodily or local conditions.

Source of vascular net of transplants. Since this phenomenon is specific for uterine tissue an attempt to discover the source of the blood vessels in the transplants seemed to be indicated. Had the blood vessels growing in from the iris anastomosed directly with the cut ends of the uterine vessels, or had an entirely new vascular net been formed? If circulation

were reëstablished in the vessels of the transplant, one should expect them to exhibit the characteristic rhythm. If, on the other hand, a new vascular bed were formed in the transplant by vessels that had grown in from the iris it had become modified so that it would blush and blanch, for the iridial vessels do not show a vascular rhythm. Two facts point towards the anastomosis of the ingrowing vessels with the original vessels in the transplant. The first is the extremely rapid vascularization of the transplants. This usually began 24 hours after transplantation and in one case complete vascularization of the transplant and the presence of the vascular rhythm were observed 20 hours after transplantation. This seems too short a time for the formation of an entirely new vascular bed and the acquisition of a rhythm by the newly formed vessels. The second fact is that in a four day transplant most of the vessels, visible microscopically, were found injected with carmine gelatine and no degenerating vessels were seen. Since the degenerating blood vessels in the transplants that fail to vascularize remain visible for more than 4 days it seems that some, if not all, of the original blood vessels were used in the formation of the functional vascular net.

Length of the rhythms. The length of the rhythm varies in different transplants in the same species. The extremes in the lengths of the vascular cycles were 15 and 25 seconds in the guinea pig, 35 and 85 seconds in the rabbit. In the 4 monkeys observed the average lengths of the rhythms were 60, 65, 75, and 80 seconds.

Microscopic structure of the transplants. After 19 months transplants of endometrium into the anterior chamber of the eyes of guinea pigs do not differ in structure from the uterus left *in situ.* Further, the microscopic structure of the transplants undergoes the same changes during the oestrous cycle as the uterus *in situ.* Mitosis are most numerous in the epithelium during the first 4 days of the oestrous cycle and in the stroma from the third to the eighth day. They are found most infrequently in the entire mucous membrane from the eleventh to the fifteenth day. The epithelium degenerates during post-oestrus but is subsequently repaired. During oestrus many leucocytes migrate out of the blood vessels of the transplant into the stroma and thence into and through the epithelium and into the anterior chamber of the eye. During oestrus the entire mucous membrane is very edematous. All these changes occur at the same time and to about the same extent as in the uterus *in situ.*

Effects of lowered blood pressure. If the blood pressure is abruptly lowered to near the lowest point compatible with life, the vascular changes in uterine transplants in rabbits' eyes are not arrested. The edges of the ears were swabbed with xylene and as soon as there was a marked dilatation of the marginal veins they were cut and from 40 to 80 cc. of blood withdrawn from each of 6 rabbits. When the large amounts of blood were

withdrawn the amount of time in partial and complete blanch was lengthened, but the greatest reduction in blood pressure obtained by this method did not greatly affect the rhythmic vascular changes.

DISCUSSION AND SUMMARY

Although cyclic vascular changes have been described in other tissues they have all been quite different in many respects from the ones found in the uterus. The evidence presented seems to indicate that these vascular changes are specific for uterine tissue, at least in a quantitative sense. If similar rhythmic vascular changes do occur in other tissues it is certain that they do so only to a far lesser extent. The facts observed indicate that the vascular rhythm is inherent in uterine tissue.

The presence of the rhythmic vascular changes in the uterus of the monkey, would lead one to suspect that they might also occur in woman. It is difficult to understand how rhythmic vascular changes in the human uterus can have escaped detection were it not for the fact that the only authors who have published descriptions of the living human uterus are Schroeder, Hinricks, and Kessler, (1926), and Schochet (1929).

The Schochet method of transplantation seems to be an especially satisfactory one, for the transplants persist without any apparent change for 23 months. This long persistence may possibly be explained by the fact that no fibrous capsule develops around successful transplants in the anterior chamber of the eye. This may be due to the absence of injury to the actual site of implantation with consequent absence of stimuli for the development of scar tissue and to the rapid removal of catabolic products by the aqueous humor which may remove stimuli to the development of a limiting capsule.

The persistence of the vascular rhythm for 30 to 60 days, in transplants, after the removal of the ovaries seems to indicate that it is not directly under the control of follicular hormone. It seems more likely that it is an inherent characteristic of uterine tissue that only manifests itself when the uterus is in an adult or reproductive state. The persistence of uterine transplants for 23 months without signs of degeneration in non-castrated males and their degeneration accompanied by the disappearance of the vascular phenomenon within 2 months after castration, seems to add evidence in favor of this hypothesis. That castration will cause the degeneration of uterine transplants in male hosts suggests that the female sex hormone described in male urine by Laqueur (1929) may be elaborated by the testicles.

These rhythmic vascular changes seem to be related to the functional activity of the gonads; appearing at sexual maturity, disappearing after spaying or castration and responding to replacement therapy.

They are also, at least temporally, related to the process of ovulation

for the period of continuous blush occurs during the final growth of the follicle.

Since the period of continuous blush can be induced by the injection of follicular hormone and since the length of the period is roughly proportional to the amount of hormone injected, it seems probable that the induction of the period of continuous blush may be used as a test of the amount of follicular hormone present in the blood stream.

Since the period of continuous blush is induced in transplants in non-castrated males by the same amount of follicular hormone as in females, there seems to be no antagonism, at least in this one respect, between follicular and testicular hormone.

The fact that two transplants in the same eye have independent rhythms seems to exclude general bodily changes as a cause.

Since pure endometrial transplants exhibit the vascular rhythm they are not caused by passive compression of the blood vessels by the contractions of the longitudinal or circular muscle of the uterus.

Modifications of the vascular rhythm seem to be governed by a hormonal rather than a nervous control.

Transplants of uterus into the anterior chamber of the eyes of guinea pigs do not become altered in structure from the uterus *in situ*, and their microscopic structure undergoes the same changes during the oestrous cycle as the undisturbed uterus *in situ*.

These rhythmic vascular changes occur in guinea pigs, rabbits and monkeys (Macacus rhesus).

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tripped at the first appearance of blood in the syringe. After removal of a control sample of blood, the measured amount of glucose was injected into the saphenous vein at the rate of 5 cc. per minute. Blood samples were removed 5, 30, 60, 90, 120, 150, 180 and 240 minutes after completion of the glucose injection. Jaundice was produced in the dogs by ligation and section of the common bile duct, plus cholecystectomy. The jaundiced animals lived for periods up to 4 months after operation. Some of the

TABLE 1

Normal dogs

WEIGHT	DOSE OF GLUCOSE	COAGULATION TIME			BLOOD CALCIUM		
		Initial	Lowest	Per cent decrease	Initial	Highest	Per cent increase
kgm.							
15.8	30 cc. of 50%	6½	3	53.8	10.7	11.7	9.4
16.0	50 cc. of 50%	10	8	20.0	11.0	12.1	10.0
15.3	50 cc. of 50%	12½	8	36.0	9.6	11.2	16.7
19.0	75 cc. of 50%	11	3	72.7	10.0	14.2	42.0
22.0	80 cc. of 50%	6	4	33.3	10.5	11.3	7.8
17.0	60 cc. of 50%	12	4½	62.5	8.0	10.4	30.0
19.1	60 cc. of 50%	10	4	60.0	9.3	10.3	10.8
20.3	80 cc. of 50%	15	3	80.0	9.3	11.0	18.3

TABLE 2

Jaundiced dogs

WEIGHT	DOSE OF GLUCOSE	COAGULATION TIME			BLOOD CALCIUM		
		Initial	Lowest	Per cent decrease	Initial	Highest	Per cent increase
kgm.							
14.4	60 cc. of 50%	13.0	4½	65.3	8.8	10.8	22.7
17.1	60 cc. of 50%	11½	9	21.7	9.1	9.8	7.9
13.4	30 cc. of 50%	18	7	61.1	9.7	11.9	22.7
12.0	60 cc. of 50%	12½	8	36.0	9.0	11.2	24.4
22.0	80 cc. of 50%	11½	5½	52.1	10.5	12.3	17.5
22.1	80 cc. of 50%	10½	4	61.9	11.0	16.4	49.0
22.1	80 cc. of 50%	11	4	63.6	11.2	14.8	32.1

animals were forcibly fed with milk and syrup to offset the anorexia and attendant marked loss in weight; others were allowed to eat only what they wished. Four dogs developed ascites and increased collateral circulation on their anterior abdominal walls during the course of the obstruction. Only one jaundiced dog had a coagulation time which was increased to any degree over the normal figure. The calcium and fibrinogen values for the jaundiced animals were within normal limits.

Table 1 summarizes the results of 8 experiments on normal dogs, and table 2 gives the results obtained in 7 similar experiments on jaundiced dogs. In all, 28 experiments were carried out.

We obtained a lowering of coagulation time after sugar injection in 76 per cent of the experiments on normal animals, and in 100 per cent of the experiments on jaundiced animals; moreover, the coagulation time was decreased 50 per cent or more in 47 per cent of the normal dogs and in 73 per cent of the jaundiced ones. These results are really quite striking. How does this increased coagulability come about? The nearly absolute parallelism between blood sugar and blood coagulability that Partos and Svec believe to exist, has not proven out in this laboratory; for the increased coagulability after sugar injection does not occur simultaneously with the increased blood sugar, but lags behind for from 15 minutes to 1½ hours after the injection. Nor does the coagulability decrease with the blood sugar, but in many cases stays up several hours after the blood

TABLE 3

Dog 12, jaundiced, weight 12 kgm., poor nutrition, not forcibly fed

TIME	COAGULATION TIME	BLOOD SUGAR	SERUM CALCIUM
	minutes	*mgm. per cent*	*mgm. per cent*
Fasting	12½	70	9.0
Injection of 50 cc. of 50% glucose intravenously in 8 minutes			
5 min.	13	136	9.7
30 min.	6	96	11.2
1 hour	8	66	10.9
1½ hour	11	74	9.6
2 hours	12	67	9.2

sugar has again returned to normal. These facts would seem to point to something other than the actual blood sugar value as the controlling factor. Ravdin reported no increase in blood fibrinogen after sugar injection. A few experiments done in this laboratory support this finding.

In a search for other changes in the blood after sugar infusions, which might explain the change in coagulability, serum calcium determinations were made. It was found that after intravenous injection of glucose the blood calcium increased with striking constancy. Furthermore, when the calcium, sugar, and coagulability values of the blood were charted together, it was seen that the calcium curve was much more nearly parallel to the coagulability curve than was the curve of the blood sugar. These results, then, throw a new light on the subject of blood sugar and blood coagulability. Table 3 shows the typical results obtained.

Discussion. It seems clear, from the above experiments, that the important effect of raising the blood sugar in the attempt to decrease the

EFFECT OF TOTAL ABSENCE OF FUNCTION ON THE OPTIC SYSTEM OF RABBITS

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The determination of the rôle that function plays in the morphogenetic development of the central nervous system is a fundamental problem that has engaged alike the attention of physiologist, neurologist, and experimental zoölogist. The optic system, due to its more or less discrete nature, has been a favorite one for experimentation on this subject. The work on mammals has been confined for the most part to the extirpation of eyes and the sewing of eyelids in an attempt to remove function from the visual pathways. Eye excision experiments have been performed by many investigators. This technic, however, is complicated by secondary degeneration, and only neurones of the third order (superior colliculus, lateral geniculate, and pulvinar) remain for study. The neurones of the first and second order (bipolar and ganglion cells, respectively, of the retina) are removed by the section of the optic nerve and cannot be examined for the effect of lack of stimulation. Sewing eyelids in rabbits (von Gudden) and in cats and dogs (Berger) does not exclude function completely since the eyelids of these animals are translucent to bright light. The very nature of these experiments, then, precludes an accurate interpretation of results. To estimate the part occupied by peripheral sensory field on the post-natal growth and differentiation of the visual apparatus in mammals, a method was sought which would leave the system under observation anatomically intact and at the same time allow the factor of complete lack of function to be the only variable.

Experimental procedure. A dark room was improvised which could be entered only through a series of three doors. Sensitive panchromatic photographic films exposed repeatedly for forty-eight hour intervals failed to record any light. This method of light determination is more sensitive than the photoelectric cell. The room was well ventilated and supplied with a large cage and a small light-proof box into which the rabbits could be put for the short time needed daily for feeding and refuse disposal. A healthy pregnant rabbit near term was placed in the room and allowed to give birth to her litter (of nine). A liberal diet containing cod liver oil was given to the mother, and to the young after weaning.

Each day the mother was removed to the sunlight for two hours, and after six weeks was taken from her young. For the duration of the experiment, six months, no one entered the room except with the writer.

When the young were nine days old and before their palpebral fissures were patent, three of the dark room animals were removed to a dimly lighted room for operation. While the right eyes were constantly well protected by a light-proof blinder attached firmly over them, the left eyes in two animals under ether anesthesia were enucleated, and in the remaining rabbit the left eyelids were sewed after the free lid margins were cut away. The procedure took less than five minutes in each instance and the animals were returned promptly to the dark room where recovery was uneventful.

The rabbits were sacrificed by asphyxia in the dark room at varying intervals. One was transferred to the light at the end of five months for a study of its optic reflexes and behavior. Three, including one of the enucleated, were allowed to remain the full six months. All animals were weighed, measured, and examined closely for evidence of deficiency disease, and the viscera and long bones preserved for study. The brains and eyes were immediately removed and fixed either in a mixture of formalin, alcohol, and glacial acetic acid, or in osmic acid and potassium dichromate for the Golgi triple silver impregnation. The brains and eyes were measured and photographed. A motion picture was taken of the behavior of the rabbit removed to the light.

Controls. A litter of rabbits born from the mating of the same buck and doe as used in the dark room experiment was raised in the light and its members killed at the same age intervals as the experimental rabbits. In addition, a number of control eye extirpation and eyelid sewing experiments were performed on very young animals raised in the light. In each case, the left side was used, and the right remained as a control since there is almost complete decussation in the rabbit's chiasm (Barker). The tissues were handled in a manner identical to that used on the dark room material.

The results of excision of an end-organ, the partial elimination of function, and the complete absence of function could thus be determined.

Gross anatomical results. In a preliminary report, the results of the first three months of the dark room experiments were given. The six month rabbits also showed excellent growth in size and weight when compared with their controls, and no evidence of deficiency disease was found. The eyes of the unoperated dark room animals presented no differences from those of the controls in size or transparency of the cornea or crystalline lens, the extra-ocular muscles, or, indeed, in any part of the accessory optic apparatus. There were no differences in the optic nerves. Careful observations and measurements of the superior colliculi, lateral genicu-

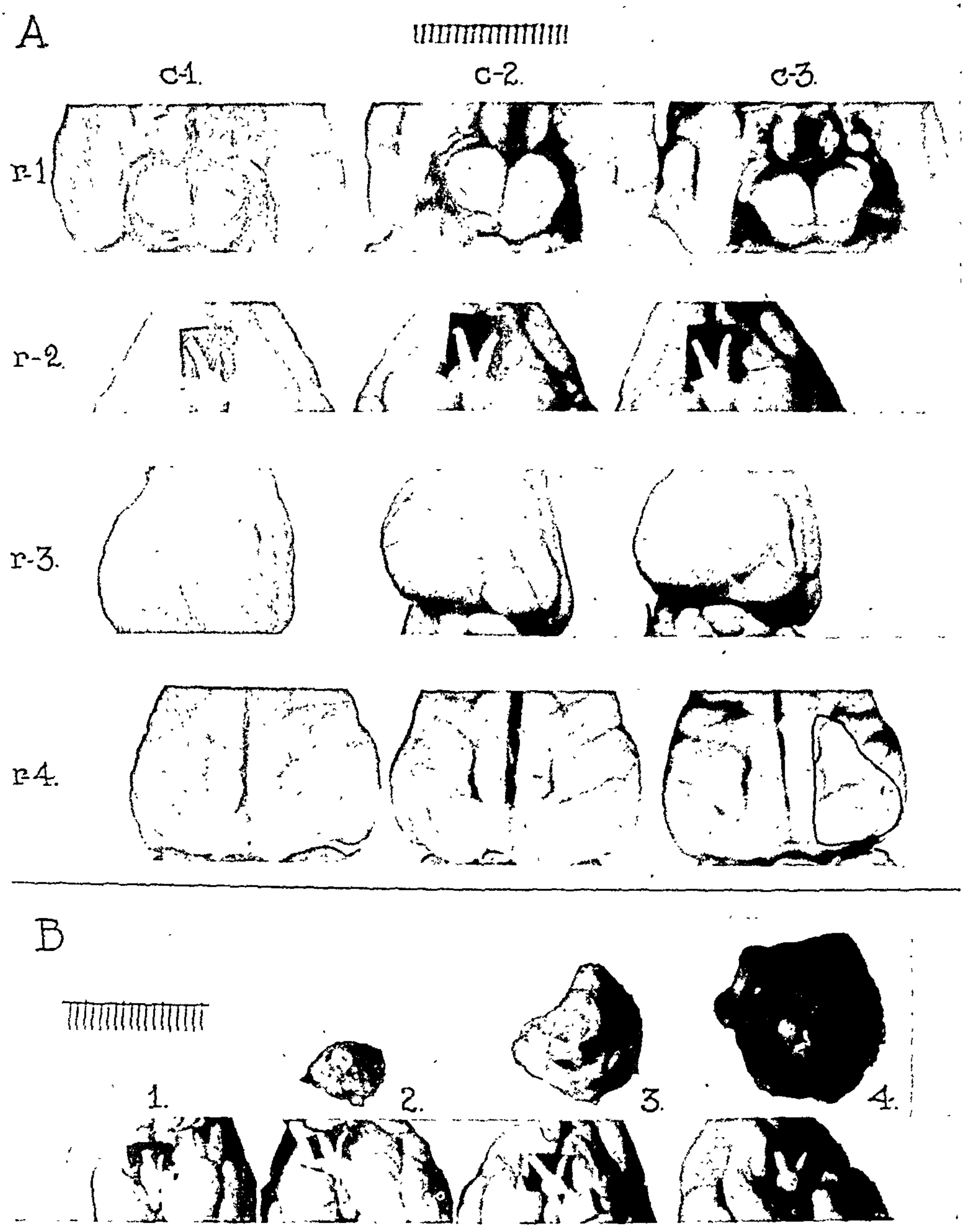

Fig. 1. Photographs of sixth month brains. *c*, column; *r*, row. A. *c-1*, dark room unilateral eye excision; *c-2*, dark room unoperated; *c-3*, light control; *r-1*, superior colliculi; *r-2*, optic nerves; *r-3*, lateral view occipital cortex; *r-4*, dorsal view. Penned area in *c-3*, *r-4* is the striate region in the rabbit. Note reduced size of the colliculus in the operated dark room animal, and the lack of cortical changes in any of the brains. Scale in millimeters.

B. *1*, Unilateral eye excision, control light experiment. *2*, *3*, and *4*, unilateral eyelid sewing light control experiments. Note relation between size of eyes and corresponding optic nerves. Scale in millimeters.

lates, and occipital cortices likewise gave no disparities not equally demonstrated in the group of normal controls (fig. 1-A, *c-2* and *c-3*).

The dark room animals with left eyes excised soon after birth (fig. 1-A, *c-1*) showed the typical degeneration changes in the cerebral stump of the left optic nerves, right optic tracts, and the right primary optic centers,—so ably described by von Gudden. No gross cortical changes were evident. The unoperated tracts could not be differentiated from the controls reared in the light (fig. 1-A, *c-3*). In these dark room enucleation experiments, then, the two procedures of removal of an end-organ and removal of function contrast sharply in the same brain. The invalidity of the former technic as a physiological method of inquiry into effects of non-use of a peripheral sensory organ on the central pathway and centers is apparent.

The dark room rabbit with eyelid sewn soon after birth was killed at the end of two months. The lid was completely ankylosed. No differences were demonstrable in comparing the eyes and brain of this animal with a two month unoperated control raised in the light. Nor were there any dissimilarities between the two sides of this experimental animal's brain.

The control eye extirpation experiments (fig. 1-B, *1*) gave the same typical von Gudden degeneration picture of the dark room enucleated rabbits. The control eyelid sewing experiments, though, differed in the following way. A series of eyes were obtained varying from one normal in size and structure (fig. 1-B, *4*) to one so scarred and atrophic that it could hardly be recognized as an eye (fig. 1-B, *2*). This is not hard to understand inasmuch as the operations were not always successful in avoiding damage to the lachrymal apparatus, and in some instances where the cornea was scarred in addition to the lid sewing, the cautery accidentally perforated into the anterior chamber. The interesting part of these experiments is that the optic nerves and superior colliculi are roughly proportional to the size of the eyes. No cortical changes were found.

MICROSCOPIC RESULTS. *Eyes and retina.* Microscopic examination of the eyes of the sixth month dark room animals showed no alteration in the structure of the cornea, lens, iris, or ciliary apparatus when these were compared with the controls.

Meridional sections of the retinae passing through the optic nerves were examined with hematoxylin-eosin, iron-hematoxylin, toluidin-blue, and triple silver impregnation stains. The total thickness of the retina and the thicknesses of the fiber and cell laminae differ considerably in the several parts of the retina, varying with the distance between the optic papilla and the ora serrata, the medullated fiber region, and the visual streak. These features of the normal rabbit's eye have been accurately described by Davis and others. Measurements of corresponding retinal

areas midway between the papilla and ora serrata and not through the visual streak or medullated fibers made with a calibrated eyepiece filar micrometer gave the following averages for several hundred determinations:

LAYER	6 MO. DARK (EXPTL.)	6 MO. LIGHT (CONTROL)
Optic fiber, ganglion cell, and internal molecular layers	37 micra	39 micra
Bipolar (int. nuclear)	17 micra	18 micra
Outer molecular	8 micra	7 micra
Outer nuclear	26 micra	27 micra
Rods and cones	24 micra	24 micra
Total thickness of retina	112 micra	115 micra

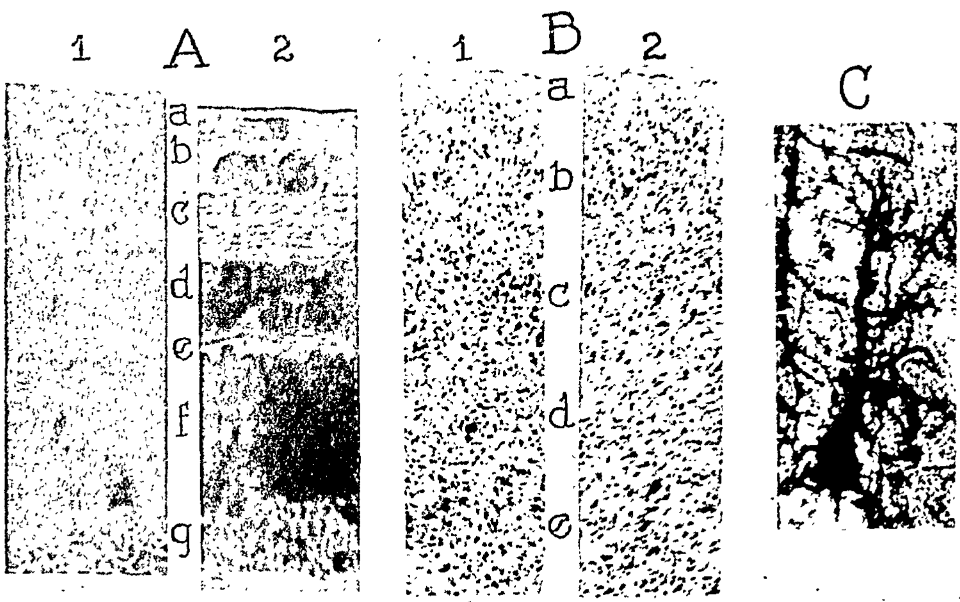

Fig. 2. Photomicrographs. A. Retinae of full term experimental, *1*, and control, *2*, rabbits. *a*, axone layer; *b*, ganglion cell layer; *c*, inner molecular layer; *d*, inner nuclear; *e*, outer molecular; *f*, outer nuclear; *g*, rods and cones, *H-E* stain.

B. Superior colliculi of sixth month experimental, *1*, and control, *2*, brains. *a*, stratum zonale; *b*, stratum griseum superficiale, outer layer of small cells; *c*, stratum griseum superficiale, inner layer of large polygonal cells; *d*, stratum medullare superficiale; *e*, stratum griseum intermedium. Note thickness and regularity of lamination. Toluidin-blue stain.

C. Small pyramidal cell of experimental striate cortex. Golgi stain.

These figures agree closely with those of Davis. Measurements through the medullated fibers and visual streak gave no appreciable differences between the control and experimental material. Also no changes were seen in the regularity of retinal layers in the experimental eyes. Photo-

micrographs of the retinal layers to show their thickness and regularity are given in figure 2-A (*1*, experimental; *2*, control).

Ganglion cell counts and measurements were made on over a hundred sections in corresponding retinal areas near the optic papilla on the temporal side, and through the visual streak. Only the larger cells with nuclei visible were measured. The results are as follows:

	AVERAGE NO. OF CELLS PER FIELD (LEITZ 5 OBJECTIVE, 10 EYEPIECE)	AVERAGE SIZE OF LARGER GANGLION CELLS
6 mo. dark (exptl.)	43 cells	15.3 micra
6 mo. light (control)	41 cells	14.7 micra

Thousands of cells of the ganglion layer were studied under oil immersion in toluidin-blue preparations for the amount and character of their Nissl material, and no alterations were found in these respects in the experi-

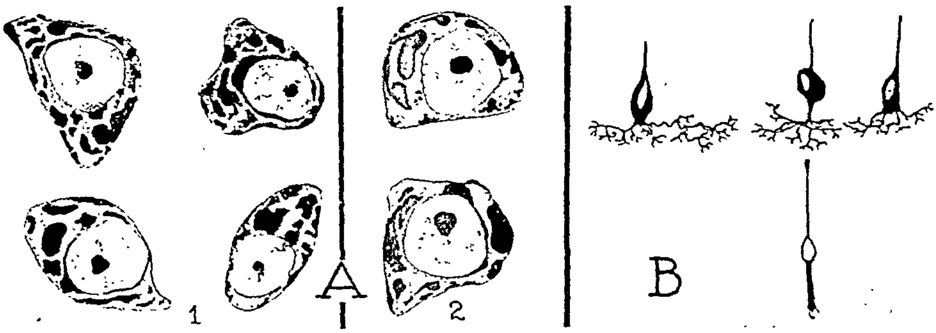

Fig. 3. Artist's drawing. Retina. A. Experimental retina ganglion cells, *1*, and controls, *2*. Note Nissl granules. Toluidin-blue stain.

B. Experimental retinal bipolar and visual cells. Note abundance of dendrites. Silver stain. See text.

mental retinae when compared with the controls. Figure 3-A shows the artist's drawing of typical retinal ganglion cells and their extra-nuclear chromatin. Many scores of Golgi triple silver impregnation preparations of the retinae were examined for the differentiation of cell processes. These as well as the iron-hematoxylin sections failed to show any difference in the degree of cell development of the experimental retinae when compared with the controls. The artist's accurate drawing of a group of bipolar cells and a visual cell (fig. 3-B) shows no lack of dendrites in the dark room retina. The axone terminals of the bipolar cells are not shown because the ganglion cell and internal molecular layers took the silver precipitate too heavily for study except at the optic papilla where the abundance of axones offered protection. In like manner, no changes were seen in the pigment layer or in the structure of the optic papilla.

The small differences noted in the tables given above are well within the percentage of error of measurement, and the differences found within each group were larger than those found between groups.

Optic nerves. Cross sections of the experimental and control optic nerves presented no dissimilarities in cross section area. Osmic acid preparations showed no changes in myelinization.

Superior colliculi. When the sixth month dark room animals were compared with their controls in respect to the regularity and thickness of cell and fiber lamination, no significant differences were found (fig. 2-B). The strata widths were measured with the micrometer in corresponding regions, taking into consideration that the relative thicknesses of the layers differ in the several parts of the colliculus, and that the architectonics are much altered by the angulation of the plane of section. Particular attention was paid to the stratum griseum superficiale (terminology of Winkler and Potter) since this is probably the most important cell layer of the optic system in the colliculus for study, receiving as it does the endings of the neurones of the second order through the optic nerve, and being mainly if not solely visual in its afferent connections. Cell counts were made on corresponding areas and reported here as number of cells per field of a micro-projector apparatus. The averages from scores of determinations are as follows:

	AVERAGE THICKNESS STRATUM GRISEUM SUPERFICIALE	AVERAGE NUMBER OF CELLS PER MICROPROJECTOR FIELD
6 mo. dark (exptl.)	870 micra	292 cells
6 mo. light (control)	850 micra	283 cells

The stratum medullare superficiale (fig. 2-B, *d*) which contains the incoming optic fibers is not as well defined for micrometer measurement. Careful study of iron-hematoxylin preparations revealed no differences, however, in this layer either in regard to the amount of fibers or their mode of distribution. Nor did the deeper cell and fiber laminae disclose any alterations when compared with the controls.

Toluidin-blue preparations for the study of Nissl granules showed the same amount and character of substance in the experimental and control colliculus cells. Figure 4-A shows the artist's drawing of the larger cells with abundant extra-nuclear chromatin. There were no dissimilarities in cell size or nucleus to cytoplasm ratio. Many excellent Golgi preparations were examined for cell processes and as shown in figure 4-B there was no lack in the experimental material of normal dendritic development.

The colliculus of the sixth month dark room enucleation experiment (fig. 5-C) confirmed on microscopic examination what the gross results

suggested. Whereas the unoperated side could not be told from the controls raised in the light, the operated side gave the changes reported in the literature by previous workers. It need only be mentioned here that as noted by Cajal the stratum zonale is unaffected; the principal changes are not in the cells (if indeed these are altered at all) but in the substantia gelatinosa of the endings of the optic nerve. These results again repudiate the interpretation of effects of sense organ removal as being due to absence of peripheral stimuli.

Lateral geniculates. In the rabbit the structure of these bodies is fairly well known except for some of the fiber connections of the ventral nucleus.

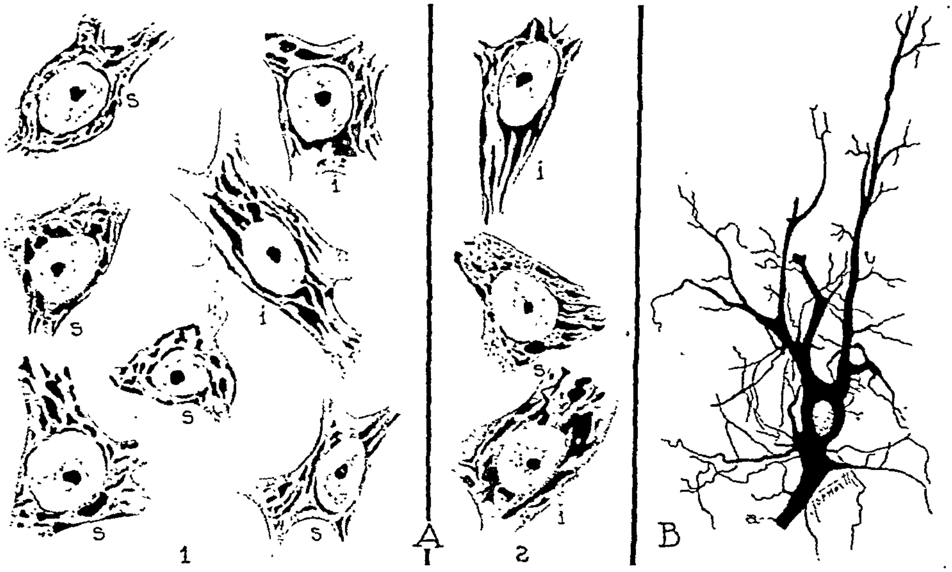

Fig. 4. Artist's drawing. Superior colliculi. A. Experimental, *1*, and control, *2*, cells. *s*, cells from superficial cell layer; *i*, cells from intermediate cell layer Note Nissl granules. Toluidin-blue stain.

B. Cell from superficial cell layer experimental colliculus. *a*, axone. Note abundance of dendrites. Golgi preparation.

They are said to receive around 80 per cent of the axones of the ganglion cells of the retina through the optic nerve and tract. The dorsal nucleus, which is thought to be the more recent phylogenetically, relays the representation of the retina to the area striata of the occipital cortex, and contains cells whose axones form the optic radiation of the internal capsule. That these cells represent neurones of the third order in the optic conduction pathway has been emphasized by Barker and others. These dorsal nuclei cells were thus considered especially important ones for this study. No discernible differences were found in the size or shape of the dorsal or ventral nuclei, nor were there any changes in the "laminations" of the

dorsal nucleus (fig. 5-A, *2*). The character and amount of Nissl substance were determined by oil immersion study of hundreds of cells of these nuclei in toluidin-blue preparations, and as shown by the artist's accurate drawings (fig. 6-A) no disparities were seen in comparing the experimental with the control sections. No differences were found in the relative num-

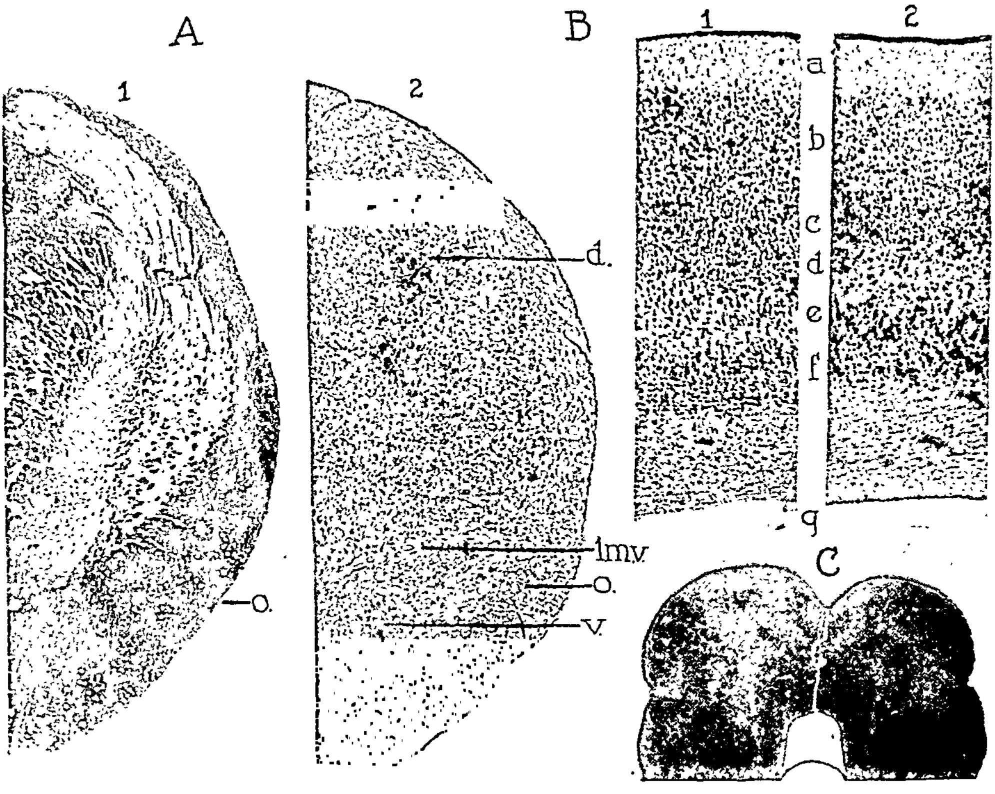

Fig. 5. Photomicrographs. A. Experimental lateral geniculate body sixth month brain. *1*, iron-hematoxylin; *2*, toluidin-blue; *d*, dorsal nucleus; *v*, ventral nucleus; *o*, optic tract on lateral aspect thalamus; *l.m.v.*, lamina medullare ventralis.

B. Experimental, 1, and control, 2, striate cortices. *a*, lamina molecularis; *b*, lamina granularis externa; *c*, lamina pyramidalis; *d*, lamina granularis interna; *e*, lamina ganglionaris; *f*, lamina multiformis; *g*, caudal tip lateral ventricle. Note thickness and regularity of lamination. Toluidin-blue stain.

C. Superior colliculi of unilateral eye excision dark room brain. Smaller colliculus on side opposite to operation. Gross specimen seen in figure 1-A, *c-1*, *r-1*, Toluidin-blue.

ber of cells or their ratio of nucleus to cytoplasm. Many Golgi preparations of this region were observed for cell differentiation and type, and here again no lack of normal dendritic development or cell types characteristic of the geniculate nuclei was discovered in the experimental material. A drawing of one of the dorsal nuclei cells stained with the silver method is shown in figure 6-B.

The dark room eye enucleation experiments, however, show a difference between the two sides of the same brain. The side whose anatomical connections were untouched could not be told from its control reared in the light. The side whose optic relations were severed by eye excision presented changes reported by other investigators. It is sufficient to state that the changes observed are mainly in the incoming optic tract fibers, the cells not being altered. This, again, as in the colliculus, shows the difference between removal of an end organ and absence of function.

Pulvinar. This diencephalic optic center likewise demonstrated no alterations when studied in the manner outlined above, although no silver preparations of this nucleus were made.

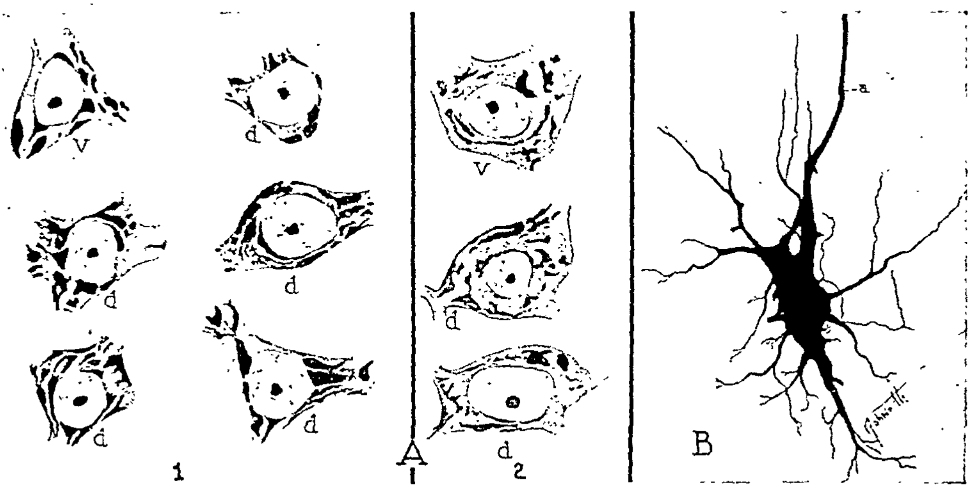

Fig. 6. Artist's drawing. Lateral geniculates. A. Experimental, *1* and control, *2*, cells; *d*, dorsal nucleus cells; *v*, ventral nucleus cells. Note Nissl granules. Toluidin-blue.

B. Experimental dorsal nucleus cell. *a*, axone. Golgi preparation.

Occipital cortex and area striata. The location and histological features of the striate area in the rabbit's cortex are well understood (Winkler and Potter; Putnam and Putnam). An examination of the thickness of the visual cortex in the experimental and control brains gave the following averages for fifty estimations made in the striate area overlying the caudal tip of the lateral ventricle:

	6 MO. DARK (EXPTL.)	6 MO. LIGHT (CONTROL)
Lamina molecularis	276 micra	282 micra
Lamina granularis externa to and including lamina multiformis	1,617 micra	1.599 micra

Similar measurements made on other representative parts of the occipital cortex showed no appreciable differences between the control and dark

room brains. A study of the regularity of lamination in the striate cortex likewise disclosed no alterations in the experimental material (fig. 5-B). Micrometer measurement of the thicknesses of each particular layer was found to be a very difficult and uncertain task, and the error too great to give dependable results. Careful examination of toluidin-blue and iron-hematoxylin preparations, however, revealed no disparities in the thicknesses of the various laminae.

Cell counts made in analogous regions demonstrated no significant differences in the number of cells in the various parts of the experimental

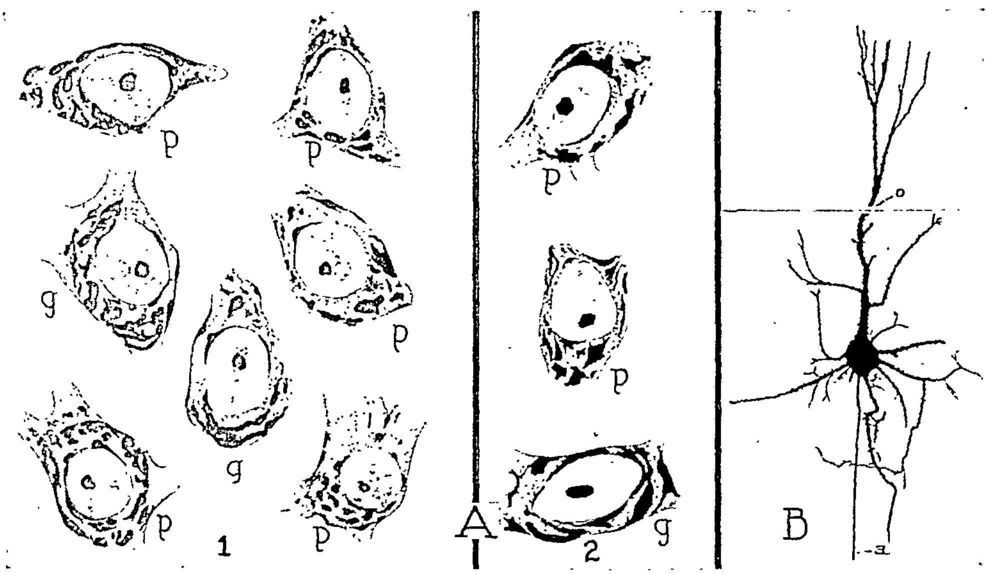

Fig. 7. Artist's drawing. Striate cortex. A. Experimental, *1*, and control, *2*, cells. *p*, pyramidal layer; *g*, ganglion layer cells. Note Nissl granules. Toluidin-blue stain.

B. Moderate-size pyramidal cell experimental striate cortex; *a*, axone; *o*, omitted portion of dendrite. Golgi preparation.

striate cortex when this was compared with the controls. One of the typical averages (ten counts) representative of numerous similar counts is as follows:

	6 MO. DARK (EXPTL.)	6 MO. LIGHT (CONTROL)
No. of pyramidal cells per field of microprojector apparatus....................................	325 cells	306 cells

No importance is attached to this difference.

Examination of the cells of the visual cortex in the experimental and control brains by means of Nissl stains showed that most of the pyramidal

and ganglion cells have large nuclei in relation to cytoplasm, and no, or fine and scant, Nissl granules. In these features of extra-nuclear chromatin and nuclear size no dissimilarities were found between the two groups. Some of the larger pyramidal and ganglion cells contain abundant and fairly large Nissl bodies and also the number of these cells do not differ in experimental and control cortices (fig. 7-A, *1* and *2*). The Golgi preparations showed normally developed dendritic processes (figs. 7-B; 2-C) and no dearth of the various kinds of cells seen in silver stains of the visual cortex. No dissimilarities were found when the experimental and control sections were compared in these respects.

A study similar to that outlined above of the cortex of the full term enucleated dark room rabbit showed no differences between the two sides. This is confirmatory of von Gudden and von Monakow.

Physiological results. A rabbit was taken into the light at the end of five months for a study of its behavior and optic reflexes. Directly before removal from the dark room its reflexes to light were tested. The first flash of light caused an immediate eyelid closure, the lid being held closed and the plica semilunaris drawn over the cornea. When the lid was forcibly opened, the pupil contracted after a latent period of five seconds.

The animal was then transferred to an open area illuminated by sunlight and strewn about with various obstacles. While in the bright light it kept its eyes almost completely shut. When in the shade, it kept its lids open. This was rather an unexpected observation that the retina could adapt itself so quickly to the intense stimulation, and allow the animal to display so little photophobia. That the rabbit was an albino lends added significance to this.

The posture of the animal was exceedingly interesting. It walked on a broad base with forelegs, especially, widely spread. Its neck protruded full length in advance of its trunk, and the head was held so low that the snout almost touched the ground.

The rabbit did not avoid obstacles placed in its path. It seemed to depend on cutaneous, olfactory, and auditory cues. It sniffed continuously, and when walking along the side of a wall would turn its head so that the vibrissae of its snout remained in contact with the wall. When placed in a low box, it manoeuvered about, feeling the edge of the box with its front feet, cautiously putting its head and neck over the edge, but not electing to jump the short distance to the ground.

When in the sunlight near a patch of shade, it did not move toward the shade; when in the shade, it avoided moving out into the sun.

For the first hour it kept its ears flat against its back. That this may have been due to fright as a result of the world of new experiences suddenly thrust upon it is suggested by the fact that its respiratory rate was fully twice as fast as that of the controls with whom its behavior was compared.

An hour later, its ears were kept erect, and its respiratory rate had diminished a third.

Its keen sense of smell was demonstrated by holding a piece of carrot several inches from its nose. At this distance the controls ignored the object, but the experimental rabbit protruded its head and walked toward it. Only when the carrot was held much closer did the controls appear to notice it.

During each of the days that followed the animal's transfer to the light, its blink, corneal, and pupillary reflexes were tested and its gait and ability to avoid obstacles were observed. In none of these respects except that of posture could the experimental rabbit be told from the controls. Within one week, its posture improved but even at the end of two months was still detectable as different. Within the first ten days, the animal advanced considerably in its ability to avoid objects in spite of the fact that it was kept all day in a very small cage. By the end of this time, even when frightened into running, it could wind in and out of numerous obstacles without hitting them. Sudden movements of objects near its eyes when due precautions were taken to prevent auditory or cutaneous stimuli from operating appeared to frighten the rabbit, and it would open its eyes widely and turn its head to the direction opposite the object (so as to bring the latter into the field of vision?).

At the end of one month the rabbit would follow with its eyes hand movements made two feet away, would turn to avoid sudden movements of objects toward him, and would jerk its head and body if the open hand was suddenly closed. Proper care was taken in all these tests to avoid other stimuli than visual.

At the end of two months the rabbit was killed and its tissues fixed, stained, and studied as outlined above. No differences, gross or microscopic, were found in any part of the optic system when this was compared with that of the control and full term dark room animals.

Discussion. The magnitude of the problem of the rôle of function in the differentiation of the nervous system makes it necessary to limit this discussion almost entirely to the more important studies in respect to the optic system. No attempt at a systematic or complete account is pretended, and many relevant and interesting data are purposely omitted.

Von Gudden after sectioning the optic nerve or destroying the retina in young rabbits observed an extremely thin optic nerve, grey instead of white, on the operated side; the opposite anterior colliculus much reduced in size; the lateral geniculate little if any changed; the cerebrum unchanged. In other experiments in which he sewed the eyelids of newborn rabbits, the gross anatomical changes were very slight. In commenting on this difference between sewing and enucleation, he stated that light probably reached the eyes through the lids, inferring that if all stimuli were removed a different picture would occur.

Von Monakow after studying the effect of eye extirpation on the primary optic centers noted that secondary degeneration never extended beyond them, and that changes in them were due to lack of molecular substance and end processes of the second nerve incident to the degeneration of the latter, and that the cells did not suffer. Cells that disappeared in the colliculus belonged to the centrifugal system. After many years, however, the main cells of the lateral geniculate showed some reduction in size, but no structural change. If the neurones of the third order are affected so meagerly by absence of the optic nerve it is hardly to be expected that they would be seriously altered by removal of impulses coming over the optic nerve.

Berger, using lid sewing on young cats and dogs, and examining them several months later, found no gross or microscopic changes in any part of the visual system except the occipital cortex. Here he noted narrower convolutions, imperfect sulci, and a thinner cortex. Microscopically there was no difference in cell numbers in the optic cortex, but a crowded arrangement of the small pyramidal cells, large nuclei in relation to cytoplasm, and fewer and finer Nissl granules. These features were interpreted as arrested development due to lack of function. He admitted that all stimulation was not removed by his procedure and accounted for the changes being found only in the cortex by assuming that the indefinite and weak stimuli are sufficient to keep the retina and primary centers normal, but that more definite and intense stimuli are needed by the cortex for development. He suggests that complete lack of light would result in marked atrophy of the optic nerve and primary centers.

The effect of eye removal, optic atrophy, and congenital micro- and anophthalmos on the cortex is a moot question. The experimental and clinicopathologic studies have not given consistent findings, and the results seem to vary with the investigator, the animal used, its age at the time of operative interference, and the length of time the experiment is allowed to continue. In bilateral enucleation in newborn rabbits neither von Gudden nor von Monakow could find any atrophy of the cortex; but the latter worker found an equal reduction of the gray and white matter diffusely throughout the occipital cortex of dogs six months after bilateral extirpation at birth. Tanzi, on the other hand, found changes in bilaterally excised young dogs relatively soon after operation, noting a decrease in size of nerve cells, and a reduction in the amount of nerve fibers and molecular substance, these changes being localized in the medial occipital convolution. The above mentioned works were done during the time that the exact localization of the visual area in the cortex was still under dispute.

Steinetz removed the eyes of larval frogs and noted the marked reduction in the size of the optic lobes after metamorphosis, showing in his figures that there was also a marked reduction in the number of cells in the opticus layers. Larsell recently repeated these experiments, enucleating eyes

unilaterally in 18–22 mm. and 30 mm. larvae of tree frogs. From more detailed cell studies he concludes that the absence of normal optic stimuli from the periphery or factors directly attributable to these stimuli accounts for the reduction in the number and size of cells, and their lack of normal dendritic development.

Donaldson described the brain of the blind-deaf mute, Laura Bridgman, and attributed the differences between the two sides of the occipital cortex to the fact that the left eye was completely blind at two years of age, but the right eye perceived light until eight. He says, "The conservation value for the nerve centers of even such weak stimuli has long been recognized and it is but natural therefore that the occipital lobe chiefly concerned with the right eye should be better preserved than the other whose development was presumptively arrested earlier during the years most important for growth." "The persistence of vision, though in a defective form, is still of great importance to the full development of the visual cortex . . ." ". . . the disturbance in the cortex is probably to be looked upon much more as due to an arrest of growth following the removal of the normal stimuli than to a continuation of the degeneration into the hemispheres." In remarking on the changes in the auditory cortex of this brain, the same writer says, "The disturbance here is most probably due to early and long continued lack of normal excitation, for the cortical cells in the sensory area are peculiarly dependent for their proper development on the special sense with which they are associated."

Workers in experimental zoölogy have studied the processes and factors involved in the proliferation and differentiation of the cells of the central nervous system. Most of this work has been performed on larval amphibia. The important contributions of Burr, Detwiler, Coghill, and many others are too numerous to enumerate here. Herrick in his review of the morphogenetic factors in the differentiation of the nervous system mentions many of these experiments and calls attention to three stages in development,—cell proliferation before maturity or function, cell differentiation or growth to functional efficiency, and a third stage in which further development may be influenced by actual transmission of nervous impulses. Burr, in an excellent short summary of the mass of difficult experimental data states that on the whole the weight of the evidence so far presented in the literature argues against the concept that functional activity is a potent factor in the morphogenesis of the central nervous system.

In regard to the experiments presented in the body of this paper, the eyelid sewing and enucleation experiments confirm the results obtained by von Gudden and von Monakow. In lid sewing, providing that the lid margins are not cut away too liberally and that no damage is done to the lachrymal apparatus so that infection, retention of secretions and pressure

atrophy do not intervene, no anatomical changes are seen in any part of the optic apparatus. The writer is inclined to interpret the results obtained in some of his lid sewing experiments (fig. 1-B) in the same manner as partial destruction of the retina or section of the optic nerve. The orbital suppuration following scarlet fever in Laura Bridgman might well account for the changes seen in her optic nerves and colliculi.

Attention is called especially to the full term dark room enucleated rabbit, as this is considered to be a most important experiment. In this animal the procedures of complete removal of peripheral sensory field and removal of sense organ were combined, and the results reported above show sharp contrast in the same brain.

It should be noted that the animal transferred to the light after five months and killed two months later showed no difference in any part of its optic system when compared with both the dark room and normal control rabbits. The fact that there was no additional growth or differentiation as a result of two months of peripheral stimulation would seem to indicate that full development was reached in spite of lack of use during the five months in the dark room. That this rabbit's lid and pupil reflexes were elicitable by the first flash of light ever to reach the retina appears to the writer to be proof that the retina and reflex centers were functional. The animal's rapid progress in its reactions to visual stimuli after removal to the light suggests that the entire system was functional and that only perceptual responses based on experience were lacking.

The present work supports Burr's summary concerning function not being a potent factor in the morphogenesis of the central nervous system. It also extends this thesis to include the post-natal development of the visual system in rabbits. Whether species other than the rabbit would give results similar to those reported here the writer is not prepared to say, nor can one predict what similar experiments on other sensory pathways might yield. The optic mechanism, being so highly specialized in function and differentiated in structure, seemed particularly favorable for study, and it was thought that if use were needed for structural development, the effects of its absence would be more striking in the optic system than in any other. The failure of such an outcome was a matter of some surprise.

It is apparent from the experiments reported in this paper that by having only one variable factor,—namely, the complete removal of peripheral stimuli without anatomical interference,—it has been possible to attribute the resulting changes, if any, to that variable and to it alone. It is not contended that peripheral sensory field plays no rôle whatsoever. It can be definitely stated, however, that the means of histological investigation and interpretation now at our disposal indicate that the lack of use has no effect on the optic pathways of the rabbit, and that the reflexes and

behavior of the animal removed to the light support the gross and cytological findings.

SUMMARY AND CONCLUSIONS

An anatomical-physiological study of the optic system of rabbits born and raised for six months in a completely dark room showed the following results:

1. There were no gross differences in any part of the eyes, optic nerves or tracts, primary optic centers, or occipital cortices.
2. Histological study of the retina in toluidin-blue, iron-hematoxylin, and Golgi silver preparations revealed no changes in thickness or regularity of layers, number or size of ganglion cells, character or amount of Nissl granules, or degree of cell process differentiation.
3. The optic nerves showed no changes in cross section area or myelinization.
4. The superior colliculi examined by the stains mentioned showed no changes in fiber or cell lamination, cell numbers, sizes, or types, nuclear size, Nissl substance, or amount of cell differentiation.
5. In like manner the lateral geniculates disclosed no alterations in the size or shape of the dorsal or ventral nuclei, the thickness or distribution of the optic tract, the number, size or types of cells, the nuclear size, Nissl bodies, or degree of cell differentiation.
6. The pulvinar presented no changes.
7. The striate cortex and cortical layers were not reduced in thickness; there were no changes in cell numbers, types, size or nuclear size, extra-nuclear chromatin, or cell differentiation.

One of the dark room animals with left eye extirpated on the ninth day after birth, and sacrificed at six months, showed in the same brain the difference between the two procedures of excision of an end organ and the complete removal of function. The significance of this particular experiment is pointed out in the text.

The lid and pupil reflexes of a dark room rabbit, removed to the light after five months for a study of its optic reflexes and behavior, responded immediately to the first stimulus of light. Within two weeks this animal showed excellent progress in its reactions to visual stimuli.

The following conclusion seems warranted: Complete lack of optic stimuli in rabbits from birth to six months of age results in no discernible changes in the visual pathways, does not prevent the lid and pupil reflexes from responding to the first stimulus of light or the animal from quickly learning to respond to visual stimuli in regulating its behavior; nor is there any indication that the presence of the peripheral sensory visual field is a factor in the development and differentiation of the optic system in rabbits, or in the ability of that system to function.

The writer wishes to thank Dr. George Burget for many helpful suggestions; and to express his deep indebtedness to Dr. Wm. F. Allen under whose direction this work was done, and whose constant advice and assistance have made these experiments possible.

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THE EFFECTS OF REPEATED ELECTRICAL STIMULATION OF THE CORTICAL MOTOR AREA IN THE CAT[1]

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Since much recent work has been done on the production of experimental convulsions by the use of convulsant drugs (e.g., absinth and camphor monobromide), I have thought that a return to the method of François-Franck in the production of clonic convulsions by electrical stimulation of the cortical motor area might serve to differentiate experimentally the direct effects of cortical excitation from the possible toxic effects of the convulsant drugs.

Cats were used in the experiments. After trephining the region over the cruciate fissure under ether anesthesia, platinum electrodes were inserted in the opening in the skull. Respiration and blood pressure were recorded. Ether was then intermitted and the experiment was begun as soon as the cortex became excitable electrically. Stimulation with a weak tetanic current was done for from two to five seconds every five minutes. The effects were those which have been recorded by François-Franck and many other observers,—an immediate clonic convulsion which persists from ten to thirty or more seconds after cessation of the stimulation. The tonic element is, however, less marked than in drug induced convulsions.

In a control series, stimulation was repeated at five minute intervals until the animals succumbed. In ten control animals, the minimal number of convulsions was thirty-seven and the maximum, fifty-two. The convulsions become less intense and of shorter duration as the experiment proceeds. Excitation of the opposite, previously unstimulated cortex at such a stage usually elicits a good clonic response except at the close of an experiment when the whole organism appears to be failing. Anatomical ablation of the region around one cruciate fissure and stimulation of the corresponding opposite side elicits clonic movements only contralateral to the intact side of the cortex.

Respiration is never so greatly accelerated as after the injection of convulsant drugs. Blood pressure falls gradually during the course of the

[1] The expenses of a part of this investigation were defrayed by a grant from the Commonwealth Fund to the Neurological Institute of New York.

experiment. Post-mortem examination usually showed a dilated stomach, general congestion of the abdominal viscera, and petechial hemorrhages in the lungs, as with drugs.

With the establishment of the general range of results in the control series, it becomes possible to use the method of electrical excitation of the cerebral cortex as a means of comparing the effects of cerebral anemia, paralysis of the skeletal musculature by curare and the administration of bromides upon the resistance of animals in which the convulsions were induced by toxic agents, such as absinth or camphor monobromide.

Cerebral anemia and cortical excitation. Anemia of the brain was induced by temporary ligation of the arteries to the head for varying periods of time and the effects of stimulation of the cortical motor area at different times were observed. After a control stimulation of the cortical motor area, with typical convulsive movements, the head arteries were occluded for from three to ten minutes and the cortical excitability was tested both during the period of occlusion and in the period of recovery.

In a series of ten animals it was found that stimulation of the cortex within one minute following the occlusion of the blood vessels produced a typical clonic convulsion; later in the occlusion period, the cortex was inexcitable. If the circulation was restored to the head within three minutes, and the general condition of the animal was good, it was usually possible to obtain a clonic convulsion on stimulation within ten minutes following the release of the head arteries. During the recovery period, stimulation of the cortical motor area caused a rise of blood pressure before the clonic convulsion could be elicited. The greatest number of occlusions combined with convulsions induced by electrical stimulation which could be obtained was six occlusions (2 to 3 minutes' duration) and ten clonic convulsions—considerably less than half the number of convulsions or occlusions obtained when done separately in control animals.

When the period of occlusion of the head arteries is longer (6 to 10 minutes) subsequent stimulation of the cortical motor area fails to produce any clonic movements at all within the time limits of an acute experiment. In acute experiments, under such conditions, tonic movements only may appear. The conditions after several days or weeks of recovery are yet to be studied.

Paralysis of the skeletal musculature by curare. It has been shown that a large part of the rise of blood pressure during the convulsive movements induced by drugs is due to the activity of the skeletal muscles (Coombs and Pike, 1931). Following the administration of absinth or camphor monobromide, the administration of curare produces a fall in blood pressure to a level from which there is no resuscitation. After a control excitation of the motor area, curare was injected intravenously and artificial respiration was maintained. Blood pressure was at first low but after about fif-

teen minutes it began to rise gradually. When it had risen to within 20 or 30 mm. of the control level, stimulation of the cortical motor area at the same rate and intensity as in the controls, was done. Although there was no observable movement of any muscle, blood pressure rose considerably more during stimulation of the motor area than in the control excitation.

In curarized animals, the number of excitations of the cortical motor area which could be done before the animals succumbed, was much greater than in the controls. The animals were still in good cardiovascular condition after 65 to 75 excitations of the cortex and the experiment usually had to be terminated either because of the exigency of the time, or the wearing off of the curare effect. In several animals in which this occurred, good clonic convulsions were obtained when the curare had ceased to be effective.

The effect of cortical excitation following administration of bromides. It has been shown that after the continuous administration of large doses of bromides for from two to three weeks, clonic convulsions do not appear after injection of absinth, but the animals succumb to lower doses of the convulsant drug than control animals (Pike and Notkin, 1931). Cats which had been fed 1.1 gram of sodium bromide daily for two to three weeks were prepared for stimulation of the cortical motor area. Using the same rate of stimulation and intensity of current which had been found to be effective in control animals, no clonic convulsions appeared in any of these cats. There were some movements of the limbs during excitation of the cortex, but these ceased as soon as stimulation ceased and were not clonic in type. Respiration became somewhat slower during the period of excitation but quickly regained its ordinary rhythm. The number of excitations possible under the conditions was greater than in the controls. After more than sixty excitations, the cats were still in good condition. At such times, when the lateness of the hour forced the end of an experiment, the intravenous injection of a low dose of absinth of camphor (0.02 cc. per lb.) caused the immediate death of the animal without a single convulsive movement. Blood pressure fell to zero and respiration ceased although the heart continued to beat for some time.

The post-mortem findings after electrial excitation as after injection of convulsant drugs are about the same—a rapid onset of rigor mortis (when there have been convulsions), dilatation of the stomach and colon, congested blood vessels of the viscera and petechial spots in the lungs being characteristic in both conditions.

Discussion. A comparison of the effects of convulsant drugs such as absinth or camphor monobromide with those of electrical excitation of the cerebral cortex brings out certain differences. First of all, the number of convulsions which the animal will withstand is from three to ten times as great after electrical excitation as after injection of convulsant drugs. Furthermore, the same conditions (e.g., curare or prolonged feeding of

bromides) which bring about an earlier demise of the animal after the injection of convulsant drugs may act to prolong the life of the animal when the cortex is excited electrically.

Although excitation of the cortical cells by whatever method elicits motor reactions through the autonomic as well as through the cerebro-spinal system, which are much the same whatever the method of excitation employed, other results seem to diverge sharply for some reason which we do not now know. These differences in the effects of the two types of convulsant agents, i.e., the physical and the chemical, must be considered in our speculations on the cause of epilepsy as it occurs clinically.

SUMMARY

1. Cats can withstand a much greater number of convulsions induced by electrical excitation of the cortical motor area than by convulsant drugs.

2. When the convulsions fail to occur because of blockage either in the cortical motor cells or at the myo-neural junction, the motor cortex can be stimulated almost indefinitely without causing the death of the animal.

3. When one side of the cortical motor area has been stimulated until there is no further bilateral clonic response, stimulation of the other side will still produce bilateral clonic convulsions.

4. After the first minute of cerebral anemia, electrical excitation of the cortical motor area is ineffective.

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AFFERENT IMPULSES AS A CAUSE OF INCREASED VENTILATION DURING MUSCULAR EXERCISE

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In the course of some observations on cardiac dyspnea, studies were made of the composition of the blood of normal men and of patients with cardiac disease before, during and after exercise (1). Except when the exertion was rather severe the changes in the acid-base state of the blood were inadequate to account for the increase in the ventilation which occurred quite regularly, even with very "mild" exercise. These findings are open to two possible interpretations: *a*, that the respiratory center is sensitive to changes in the blood which are too small to be detectable by the methods used, or *b*, that the increase in ventilation occurring under such conditions is due to some factors other than alterations in the acid-base condition of the blood.

The first of these two hypotheses seemed to us unlikely because in several instances the blood taken at the time when the ventilation was greatest was actually somewhat more alkaline than when the subject was at rest. Furthermore, additional experiments were done (2), and it was shown that dogs required a relatively large alteration in the acid-base state of the blood to produce changes in ventilation comparable to those occurring in man after slight exertion.

It seemed therefore that the explanation for the increased ventilation produced by mild exercise had to be sought elsewhere. The following experiments are the result of such an investigation.

Results. A. *Observations on man.* Ventilation was measured either with a Benedict or with a Tissot spirometer. After the breathing had been recorded for several minutes in the resting state the subject was told to open and close the hands at a rapid rate. This exercise was performed for one minute. It was found that opening and closing the hands led to a slight increase in ventilation, and this occurred whether or not the circulation to and from the moving parts was impeded by a blood pressure cuff placed around the proximal portions of the arm and inflated to 200 mm. pressure. Such observations which are illustrated in figure 1, seemed to indicate that the increase in ventilation produced by this muscular effort could not be due to any chemical alteration in the blood induced by the muscular activity and suggested that the effect on respiration is due to "nervous" influence.

Theoretically, such a stimulation of breathing by means of the nervous system might be reflex, or it might be due to the irradiation of motor impulses from the cerebral cortex to the respiratory center (Krogh and Lindhard, 3). In figure 1 it is shown however that passive movements were likewise associated with increase in ventilation which occurred whether or not the circulation was intact. This finding suggests that the effect was of reflex origin and is in accord with the later observations of Krogh and Lindhard (4) in regard to passive movements.

Since the circulation of the bones was not obstructed it might be argued that the increase in ventilation was due to chemical changes in the blood passing to the general circulation by way of the bones. In order to settle this point further observations were made.

B. *Experiments on dogs.* The animals were anesthetized with barbital. Respiration was recorded in some instances, by allowing the dog to rebreathe oxygen from a Benedict spirometer. In other experiments, when accurate measurements of the ventilation were desired a different method

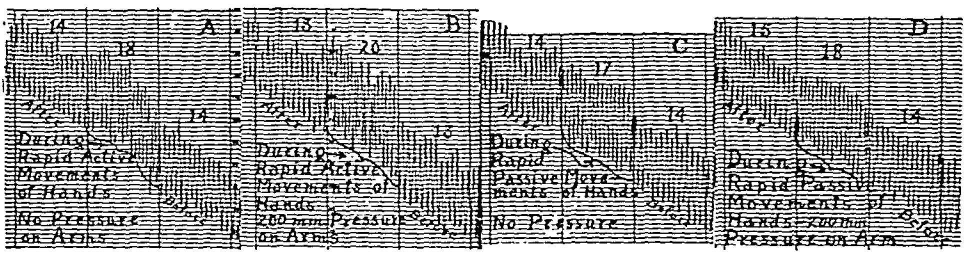

Fig. 1. The curve runs from right to left. The numbers refer to respirations per minute. The distance between the vertical lines represents one minute.

was used, the expired air being collected in a small spirometer. Passive movements were performed at a rate of about 300 per minute, an effort being made to avoid moving any parts of the body other than the extremity in question. This was not entirely possible in animals with intact extremities.

1. *The effect of cutting the spinal cord on the respiratory response to passive movements.* One animal showed a definite although slight response to movements of the hind-leg and of the fore-leg when the spinal cord was intact. After chordotomy, at the level of the sixth dorsal vertebra, the respiration was not affected by passive movements of the hind-leg but became accelerated when the fore-leg was moved.

In another animal (fig. 2) more striking results were obtained. When the spinal cord was intact well marked effects were obtained by moving, in order of increasing response, the tail, the hind-leg, the fore-leg and the head. (In the figure, for the sake of convenience in labelling, "leg" refers to the hind-leg and "arm" to the fore-leg.) It was noted that the

mass of muscle being moved was least when the tail was moved, more with the hind-leg, still more with the arm and most with the head. After the spinal cord had been cut at the fourth dorsal level passive movements of the fore-leg and of the head gave the same response as before, whereas movements of the hind-leg and of the tail were without effect.

It should be emphasized that such negative results were only obtained when the spinal cord was cut above the level of the nerve supply of the moving muscles. Thus, cutting the spinal cord at the ninth or tenth dorsal level did not always abolish the effect of moving the hind-leg because such movements were associated with slight movements of the abdominal muscles. However, cutting the spinal cord in the upper or middle dorsal region invariably abolished the respiratory response to movements of the hind-leg.

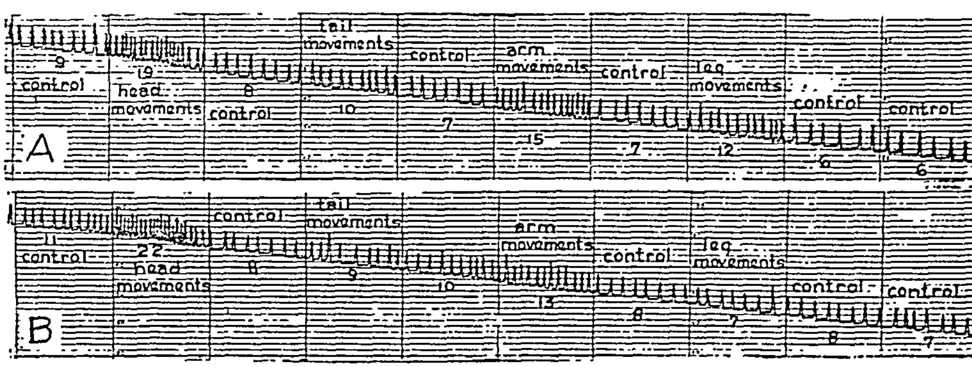

Fig. 2. The curve runs from right to left. The numbers refer to respirations per minute. The distance between the vertical lines represents one minute. A, record with spinal cord intact; B, record after spinal cord was cut at fourth dorsal level.

2. *The respiratory response to passive movements of legs completely amputated except for the femoral vessels and the sciatic nerve.* To the experiments on both men and dogs reported up to this point, two objections can be raised. In the first place since the bones were intact there is the possibility of the results having been influenced by blood returning from the moving extremity. In the second place, it seems very likely that the respiration was affected by motions of the muscles of the buttocks and of the abdomen. Although the data which have been presented point toward such an effect being reflex it might conceivably have been chemical as it was not possible when dealing with intact extremities to move them without causing some movements higher up and in no case was the venous return obstructed from these more proximal muscles.

In order to meet these objections the leg was amputated at the hip joint and all connections between the leg and the trunk except the femoral vessels and the sciatic nerve were severed. The femur was then tightly

clamped in a vise, in such a position that neither the vessels nor the nerves were under tension. This procedure had the double advantage of making it possible to isolate the chemical from the nervous effects and also allowed one to make vigorous passive movements of the leg without causing any movement in the muscles proximal to the leg.

The results are illustrated in figure 3. It was found that even when the the nerve and vessels were intact the effects of passive motions were rather slight, being usually much less than in the previous experiments. This is to be attributed to the shock which necessarily ensues following amputation, and also to the fact that the mass of moving vessels was less in the amputated than in the intact legs. Despite the smallness of the effect the increase in ventilation occurred in each experiment. (The maximum error of measurement with the small spirometers used is not greater than

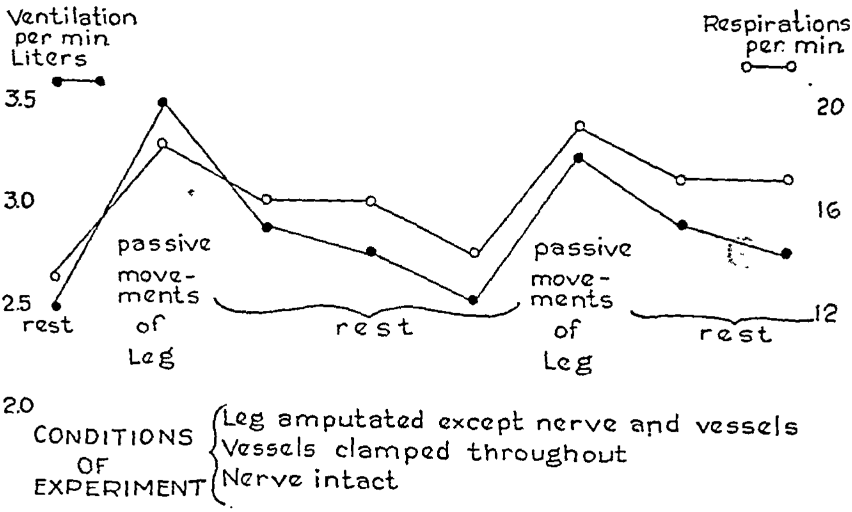

Fig. 3

0.04 liter and in no instance was the increase in ventilation on passive movements less than three times possible error.)

Following these observations the femoral vessels were occluded with bull dog clamps and the procedure was repeated. Again, in each of the experiments an increase in ventilation resulted, although in one of five animals this was scarcely greater than the error of measurement.

The clamps were then removed from the vessels, the sciatic nerve was cut, and after waiting a few minutes for the respiration to become constant, the procedure was repeated for the third time. In one experiment of the five a slight increase in ventilation resulted. In the remaining four instances moving the leg, when the sciatic nerve had been cut, was without effect even though the blood supply was intact.

3. *The rapidity of the response of the ventilation to passive movements.*

As can be seen in the figures the increase in breathing usually occurred immediately after the beginning of passive movements. This fact is probably the most convincing evidence obtained that the effect was of reflex rather than of chemical origin.

4. *The return of the ventilation to normal after the cessation of passive movements.* In instances in which the increase in ventilation was of small degree the respiration often returned to the previous control level as soon as the movements ceased. However, when a larger increase in ventilation occurred there was sometimes a "hang-over" effect, the respiration gradually returning to normal over a two or three minute period. This phenomenon was rather puzzling and led us to suspect at first that there might be a delayed chemical effect by means of alteration in the blood as well as an immediate reflex effect. However, experiments such as those portrayed in figure 3 showed that this was not the case. This animal showed a definite increase in ventilation for at least two minutes after the cessation of the movements. Since there was no possibility in this instance of any blood from the leg returning to the general circulation it is obvious that the delayed as well as the immediate increase in ventilation was due, in this instance at least, to nervous influences.

On second thought this did not seem surprising. It has been shown by Garrey (5) that stimulation of a nerve ganglion produces an increase in its metabolism, i.e., produces chemical changes in the ganglion. Hence, one might expect a delayed return of the respiration to normal for time might be required for reversal of the chemical changes induced by stimulation of the respiratory center, whether by reflexes or by other means.

SUMMARY. 1. Active movements of the extremities of man cause increased ventilation.

2. The result is not affected by the presence of an inflated blood pressure cuff proximal to the moving parts of the extremity.

3. Passive movements likewise increase the breathing of both man and dog.

4. Cutting the spinal cord of dogs abolishes the effect.

5. If the posterior extremity of a dog is severed from the body except for the femoral artery and vein and the sciatic nerve, movements of the extremity cause increase in ventilation regardless of obstruction of the blood vessels. Movements of the extremity are not associated with increase in ventilation if the sciatic nerve has been divided.

CONCLUSION

The increase in ventilation during muscular exercise, which is not severe enough to cause detectable alteration in the oxygenation or the acid-base condition of the blood, is due—in part at least—to respiratory reflexes arising in the moving parts.

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THE EFFECT OF METHYL, PROPYL AND BUTYL ALCOHOL ON THE GROWTH OF WHITE LEGHORN CHICKENS

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The aim of this investigation was to determine the effect of the administration of different concentrations of methyl, propyl and butyl alcohol on the growth and vigor of White Leghorn chickens.

METHODS. The alcohols were used in two concentrations, light and heavy. The initial concentration and dose were determined by experiments based on the fact that the anesthetizing power of the straight chain alcohols is proportional to their molecular weight. Various amounts of different concentrations of the alcohols were injected into the crops and the dosage chosen that brought on the primary symptoms of intoxication—staggering with occasional tumbles.

Ninety-five day old chicks were divided into groups as follows: 15 for each of the heavy alcohol groups, 14 for each of the light alcohol groups and 8 for controls which were given daily doses of water on the same schedule as that of the light alcohols.

The doses were administered each morning between six-thirty and eight o'clock by means of a graduated pipette, starting with a one cubic centimeter and ending with a twenty-five cubic centimeter pipette. These were inserted deep down into the esophagus, the liquid being released into the empty crop at a moderate rate of speed. If the pipettes were not inserted deep enough or the liquid allowed to run out too rapidly, the material would dam up in the esophagus and some of it enter the glottis causing irritation of the lining tissues and bringing about a condition of edema.

DOSAGE. The light methyl group received the following dosage: for the first 3 days, 0.25 cc. of 20 per cent; the next week, 0.5 cc.; the third week, 0.7 cc.; the fourth week, 0.7 cc. of 25 per cent which was gradually increased until at the end of two months they were receiving 2.5 cc. and at the end of 16 weeks most of them 12 cc. The heavy methyl group was treated as follows: for the first 3 days, 0.25 cc. of 40 per cent; for the next week, 0.3 cc. and the next, 0.4 cc.; this was gradually increased until at the end of two months it amounted to 1.5 cc. The concentration was then increased to 45 per cent and at the end of the third month they were

receiving 2.5 cc.; this was increased until at the end of the experiments the fowls were receiving from 5 to 9 cc. according to individual differences.

The initial dosage of heavy propyl alcohol was 0.25 cc. of 12 per cent, after 4 days 0.3 cc. was given, 6 days later 0.4 cc., at the end of 2 weeks 0.5 cc.; at the end of 3 weeks, 0.7 cc. At one month they were given 1 cc. of 13 per cent; at 2 months 2 cc. of 14 per cent; at 9 weeks 2.5 cc.; at 10 weeks, 2.5 cc. of 16 per cent; at 11 weeks, 3 cc.; at 12 weeks, 4 cc.; at 13 weeks, 4.5 cc. of 18 per cent. At 16 weeks they were receiving 6 cc. of 18 per cent. The light alcohol chicks were treated on the same schedule with one-half the concentration.

Butyl alcohol, with the greatest molecular weight, proved the most potent. The heavy alcohol group was treated as follows: for the first 3 days, 0.25 cc. of 6 per cent; for the next 4 days, 0.3 cc.; for the next 3 days, 0.4 cc.; at 2 weeks 0.6 cc.; at 18 days, 0.7 cc.; at 3 weeks, 0.8 cc.; at one month, 1 cc. of 7 per cent. At 10 weeks they were given 5 cc. of 8 per cent; this was gradually increased until at the end of the experiment they were receiving 7.5 cc. of 8 per cent alcohol. The light butyl group was injected on the same schedule with one-half the concentration.

Results. The growth of the four different lines is shown in table 1. All the alcohols appeared to have a somewhat stimulating effect on growth during the first three weeks, but by the fourth week the heavy propyl group began to fall behind. They made consistently smaller gains than the other chicks and were considerably emaciated at the end of the experiment. At the age of 6 weeks the heavy methyl group began to lag, and at 9 weeks the same was true of the light propyl chicks.

If the sum of the average of the males and the average of the females in each group is divided by two we obtain the following final weights in grams: controls, 922; light methyl, 864; heavy butyl, 859; light butyl, 827; light propyl, 745; heavy methyl, 657; heavy butyl, 512. In other words, the two butyl and light methyl groups finished within 90 to 96 per cent of the weight of the controls; the light propyls at about 80 per cent, the heavy methyl at 71 per cent and the heavy propyl at 55 per cent.

The feathers of the chicks in the heavy methyl alcohol group were dull colored, ruffled and comparatively sparse; the muscles were flabby so that the wings generally dropped. The skin was pale and the combs small and anemic. These conditions were not so marked in the light alcohol group but were present to some degree. With five of the heavy methyl group abscesses formed in the wall of the crop. These plugs would fall out leaving a small hole which quickly healed. Macroscopic examination of the viscera of several of the birds failed to show any further abnormalities. These birds were nervous, especially the heavy alcohol

group. There was no evidence of the effects attributed to the drinking of this alcohol by man—blindness, deafness and paralysis.

There were more fatalities in the heavy propyl group than in the others, four of the chicks dying, because of the tendency of this drug to effervesce when injected into a crop which contained mash, causing an eruption of the material up the esophagus into the glottis and the consequent strangling of the chick. In this group the tails were sparsely feathered, the combs small, the feathers not compact nor sleek looking, while the skin and legs showed a tendency towards anemia. The same facts were true in regard

TABLE 1

Average weekly weight in grams

AGE	CONTROLS		LIGHT METHYL ALCOHOL		HEAVY METHYL ALCOHOL		LIGHT PROPYL ALCOHOL		HEAVY PROPYL ALCOHOL		LIGHT BUTYL ALCOHOL		HEAVY BUTYL ALCOHOL	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
	Number of chicks at the end of the experiment													
	5	3	9	5	7	5	6	7	3	6	7	7	8	7
weeks														
0	39	39	36	35	35	36	37	35	36	35	36	36	37	36
1	53	50	47	47	56	50	47	57	50	48	47	53	61	52
2	71	70	73	81	89	75	73	79	76	66	76	86	83	80
3	103	105	107	124	138	109	119	121	111	102	120	128	121	132
4	143	140	142	160	178	142	159	154	144	126	158	192	172	167
5	191	193	184	220	207	175	200	188	178	162	192	206	204	201
6	271	261	263	263	231	194	261	239	229	209	262	272	263	251
7	324	295	312	325	297	223	337	297	298	243	321	346	349	341
8	385	354	369	358	354	273	377	315	338	265	377	388	383	374
9	447	421	427	413	414	313	407	350	341	277	435	450	433	421
10	518	488	483	468	458	332	481	396	367	314	480	497	511	479
11	562	515	523	513	480	349	536	442	401	334	528	537	576	530
12	626	565	581	541	583	387	598	479	422	358	574	582	616	574
13	720	652	676	597	622	402	665	507	436	383	627	643	692	648
14	827	700	766	663	744	443	700	541	488	415	679	700	766	707
15	909	752	841	719	761	472	757	592	511	441	756	744	848	735
16	1,012	832	959	768	841	472	868	622	553	471	856	798	932	786

to the light propyl group but to a less degree. There was a comparatively large number of hyper-irritable chicks in the propyl group, this being especially true among the heavy alcohol chicks.

Butyl alcohol affected the chicks the least of all, both as to weight and appearance. Almost the only difference between these birds and the controls was the anemic condition of most of the combs and the pale legs of the alcoholized chicks. On the whole this group was the tamest of all the fowls.

In a previous investigation (1930) light doses of ethyl alcohol had a

favorable effect on the growth and appearance of the chicks, this group outgrowing the other fowls and also having larger, redder combs and brighter, more compact feathers. The group receiving heavy doses of ethyl alcohol were more excitable than the others, while their feathers and combs were dull in color. The average weight attained at 16 weeks when males and females were averaged together as mentioned above was: light alcohol group, 982 grams, controls, 923 grams; heavy alcohol group, 856 grams.

SUMMARY

1. Heavy doses of methyl alcohol injected into the crops of growing chicks had an injurious effect on the growth and vigor as judged from appearance and also the disposition. Abscesses formed in the crop walls of a few of the subjects.

2. Light doses of methyl alcohol had a slight effect on growth, but influenced unfavorably the feathering, combs and disposition of the chicks.

3. Heavy doses of propyl alcohol had a markedly injurious effect on growth, and a somewhat deleterious one on appearance and disposition.

4. The same was true of light doses of propyl alcohol in a lesser degree.

5. Both light and heavy doses of butyl alcohol did not retard the growth of the chicks nor affect the appearance, except that the combs were somewhat anemic.

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EXTRAOCULAR REFLEXES

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The experiments reported here were instigated by the difference of opinion concerning receptors for the stretch reflex expressed in two papers appearing in 1928. The first by J. F. Fulton and J. PiSuñer, concluded that muscle spindles excite the reflex and suggested that tendon spindles probably inhibit it. The second, by D. Denny-Brown, assigned the excitatory rôle to the tendon receptors, the inhibitory to the muscle spindles. The extraocular muscles of the cat contain neither of these end organs (Cilimbaris, 1930) and hence should afford an instructive comparison with the extensors of the lower limb of the same animal studied in these two researches. Had we realized that P. Hoffmann (1929) had already reported the absence of the stretch reflex in extraocular muscles, or could we have anticipated the identification of the muscle spindle with the end organ responding to passive stretch by B. Matthews (1931), we should not not have undertaken the present work. Yet it seems worth reporting because it affords details of reflex patterns not previously described which bear upon the vexed question of the origin of the quick component of vestibular nystagmus.

METHOD. Cats were decerebrated under ether anesthesia, by the trephine method after inserting a tracheal cannula and looping the carotid arteries for temporary occlusion. Transection passed through or just above the superior colliculi and oral to the exit of the 3rd nerves. In case of transection between medulla and cord, this was done at the time of decerebration. The cartilaginous outer wall of one orbit was then excised; all extraocular muscles were identified by labelled silk ligatures, tied at their insertions, and freed from the globe, which was enucleated. In several instances a small patch of sclera was removed with each muscle to avoid injuring receptors at the insertion. Since such preparations showed no deviation in response, the practice was discontinued. The head holder was fixed firmly by a bar in a slot through the barrel of a microscope the base of which was screwed to a heavy block clamped to the table. In recording responses to stretch, the head was dropped by the coarse adjustment of the rachet and pinion of the microscope, the extent of drop recorded by one lever of the myograph attached to a drill hole in the bony orbit.

In the last two experiments a platform on which the cat was lying was moved in this manner, thus avoiding twist of the neck and hence the need of suboccipital transection. A frictionless double torsion lever recorded optically on the shadowgraph principle. For the last two experiments it was modified to a mirror principle. Early in the research the sensitivity was greatly enhanced by grinding down the torsion elements. After this modification the vibration frequency fell to 100 d.v. per second. Figure 1 shows records obtained prior to this change. With rare exceptions (fig. 2c and d) the recorded muscle was attached to the distal end of the lever shank by silk thread. Under these conditions, shortening approximates 1 mm. per gram. The tensions developed were so low that we do not believe the records were seriously distorted through stretch of the thread. This opinion is based chiefly upon the form of our records of submaximal neuromyal tetani some of which are shown in figure 2.

In recording from the recti muscles in reflexes such as the retraction associated with the protective wink, in which the entire retractor bulbi contracts powerfully, it is essential to distinguish between active contraction of the recti and their passive transmission of the pull from retractor bulbi. Our attempts to section the nerve supply as it enters a muscle without injury to the remainder of the nerve were unsuccessful. Instead, the retractor bulbi was tied out in isometric fixation. Of the three methods employed for this purpose, that which interfered least with the recording recti was to insert the thread from each muscle in a slit in a small wooden peg which was plugged into a hole in a brass bar fastened horizontally above the preparation. The pegs were then twisted, winding the threads to the desired tension in the manner of keys in tuning a stringed instrument.

RESULTS. *Stretch.* The response to stretch was tested in twenty-three experiments in which controls were obtained after section of the motor nerve. Eleven of these were in animals with nervous system intact below the level of decerebration (six testing external rectus, two inferior oblique, three retractor bulbi). In one experiment on internal rectus, the only additional feature was suboccipital transection to eliminate neck reflexes. In eleven experiments (ten of them on external rectus) the labyrinths were silenced by either section of the eighth nerve or cocainization by the method of de Kleyn (Magnus, 1924). In six of them (five on external rectus, one on internal rectus,) the ipsilateral trigeminal nerve was cut. In nineteen experiments the change in tension was identical with that in controls after motor nerve section. In four, the tensions developed slightly exceeded those of the controls (fig. 1). Three of these were in external rectus, one in the superior external head of retractor bulbi. In none of these apparently positive experiments were reflexes from neck and labyrinth blocked. In several of the animals in which these possible sources of error were eliminated, permanent loss in tone of extraocular muscles was observed to follow

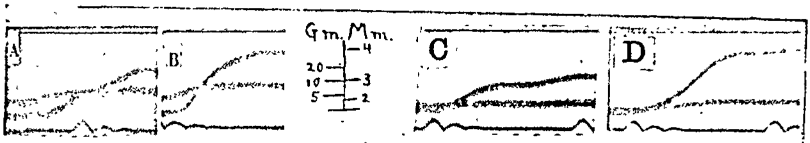

FIGURE 1

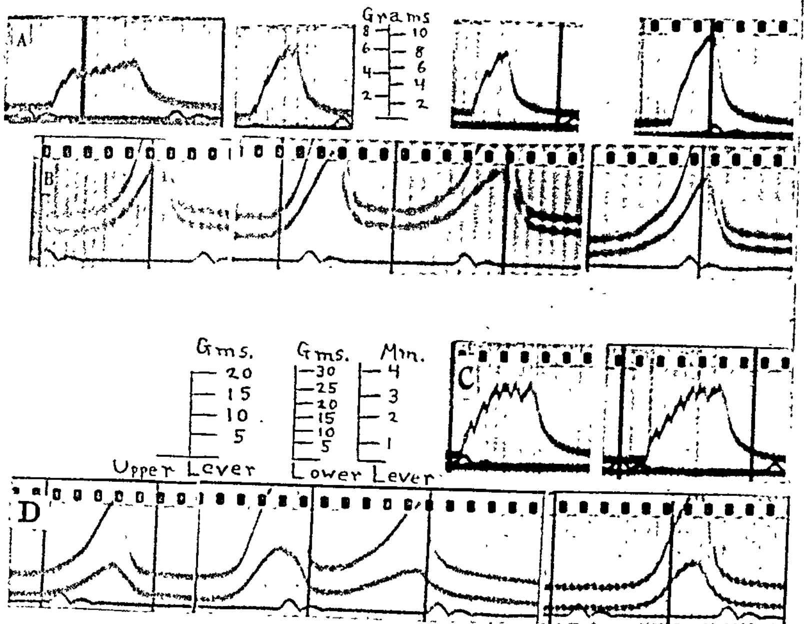

FIGURE 2

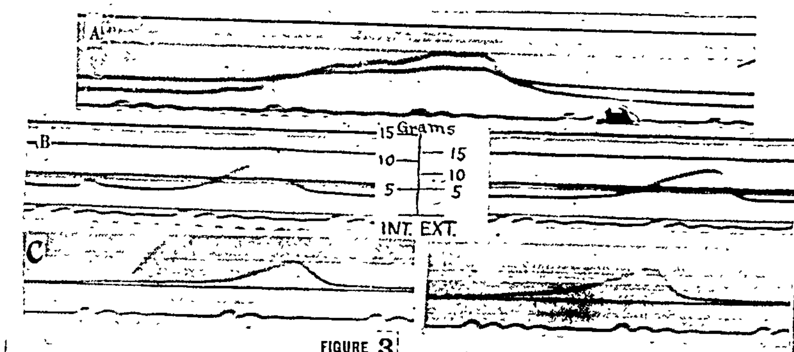

FIGURE 3

Fig. 1. Stretch of external rectus. Experiment of 1/25/29. A and B before, C and D after section of abducens nerve. The lever with the larger excursion indicates drop of the head. This is the greatest difference between experimental and control records obtained in the entire series. In all records lowest line indicates time in 0.2 second.

Fig. 2. A and C: Submaximal neuromyal tetani of internal rectus. Experiment of 5/8/29. B and D: Retraction reflex from stimulation of conjunctiva. Experiment of 4/24/29. Rectractor bulbi: Superior external head above; inferior internal head below. C and D are recorded more isometrically than A and B.

Fig. 3. Retraction reflex from stimulation of conjunctiva. A and B: External rectus above; internal rectus below. Experiment of 6/23/30. In A, the retractor is free; in B it is tied in isometric fixation. C: Gradual onset of recruitment in weak reflex. Experiment of 9/12/30. Inferior internal head of retractor bulbi. Standardization for external rectus in A and B is valid for C.

section of the eighth nerves or cocainization of the labyrinths. In no experiment could a jerk response be elicited by tapping the head holder.

Retraction reflex. In some of the experiments on stretch, occasional, brief contractions of considerable magnitude were observed which were concerned with retraction of the eye during the protective wink. These could be elicited in any preparation in good condition by touching the conjunctiva near the inner canthus. In some instances the conjunctiva of the other eye, or even the nasal mucosa proved an adequate field for eliciting the response. This reflex was studied in twenty-one experiments. No trace of it was observed in either superior or inferior oblique. All of the recti share in it passively, transmitting the pull of the strongly contracting retractor bulbi. The passive nature of this response was demonstrated in the case of superior and inferior rectus by the fact that it was not altered by section of the oculo-motor nerve. It was frequently abolished in all of the recti by isometric fixation of retractor bulbi. On the other hand, one or more of the recti often share actively in the contraction. Of the six experiments in which internal and external rectus were synchronously recorded, with fixation of retractor bulbi, internal rectus contracted in two, external in two, neither in two, though in all six both muscles showed the usual passive response with the retractor released (fig. 3). In one of the animals with active participation of internal rectus, it was noted that, prior to enucleation, the retraction was associated with internal rotation.

The form of the tension curve is invariably sigmoid. Recruitment is usually rapid (from 100 σ to 150 σ) and is often interrupted before attaining a plateau by relaxation almost as rapid and complete as that following a neuromyal tetanus (fig. 2). When the reflex is weak, however, it may recruit for as long as 300 σ, hold a plateau for 70 or 80 σ and relax at a declining rate for 200 σ to 300 σ. Such a sluggish wink may show three or four undulations in the course of recruitment (fig. 3). With isometric recording external rectus may develop a tension of 40 grams. With the considerable shortening permitted in most of our experiments, tensions of 10 to 20 grams are more common. Individual heads of retractor bulbi often show similar tensions; but in powerful retractions they may develop tensions which carry the lever shadow off the film (over 40 grams).

Our crude mechanical method of stimulation (touching the conjunctiva with a blunt muscle hook) does not permit determinations of true latency. Yet there can be no doubt that the duration of latent summation is extremely variable. Preparations in good condition responded to touch too light to give an artifact upon the record. Others in which it may be presumed the innervation of the conjunctiva was more damaged by the enucleation, often received strokes which pulled upon the tissue within the orbit sufficiently to induce a mechanical artifact. Such records indicate

that latent summation may continue as long as 240 σ before inducing a response and durations of 150 σ are common.

Nystagmus. In the experiments on stretch, the condition of the center was frequently tested by caloric vestibular nystagmus. "Spontaneous" nystagmus also occurred frequently in our records. Its vestibular origin is indicated by its permanent cessation after cocainization of the labyrinths.

TABLE 1

Influence of stretch on nystagmus

NUMBER OF BEATS MEASURED	EXPERIMENT OF 1/25/29	DURATION IN σ: Slow component	DURATION IN σ: Quick component	SLOW/QUICK	TENSION: Trough	TENSION: Peak	TENSION: Tensile change per beat	STRETCH
					grams	*grams*	*grams*	*mm.*
7	Average	422	125	3.4	6.7	10.4	3.7	4.0
	Maximum	528	150	4.2	6.7	11.0	4.0	4.0
	Minimum	360	102	2.4	6.0	10.0	3.0	4.0
8	Average	379	137	2.9	4.0	6.4	2.4	0
	Maximum	460	150	3.5	4.0	7.0	3.0	0
	Minimum	312	120	2.2	4.0	6.0	2.0	0
	EXPERIMENT OF 4/3/29							
7	Average	348	242	1.4	1.0	3.0	2.0	0
	Maximum	378	276	1.7	1.0	3.0	2.0	0
	Minimum	330	222	1.3	1.0	3.0	2.0	0
6	Average	324	249	1.3	3.0	5.8	2.8	4.7
	Maximum	348	276	1.5	3.0	5.8	2.8	4.7
	Minimum	306	228	1.1	3.0	5.8	2.8	4.7
5	Average	343	269	1.3	1.5	3.5	2.0	1.0
	Maximum	372	288	1.5	1.5	3.5	2.0	1.0
	Minimum	324	252	1.2	1.5	3.5	2.0	1.0
7	Average	296	246	1.2	3.0	6.0	3.0	5.0
	Maximum	336	264	1.3	3.0	6.0	3.0	5.0
	Minimum	270	216	1.1	3.0	6.0	3.0	5.0

The pattern of this reflex contrasts with the retractor response in both distribution and course within the individual muscles. The change in tension per beat rarely exceeds four grams and is frequently less than one gram. Figure 4 shows a slow, strong, "spontaneous," lateral nystagmus. The myograms of internal and of external rectus are not mirror images of each other. Contraction develops by recruitment at an almost constant rate. Relaxation begins more abruptly, terminates more gradually. In strong,

rapid nystagmus contraction approaches more closely to a d'emblée response. Yet even here relaxation is more abrupt in onset. Duration ratios of $\frac{\text{slow}}{\text{quick}}$ components vary within a single experiment from 1.6 to 7.2. Sudden stretch or release from stretch by dropping or raising the head from one to five millimeters has no consistent observable effect upon the rhythm and no more upon the tensile change than it would induce in a neuromyal preparation (table 1). In one experiment (1/25/29) the slow

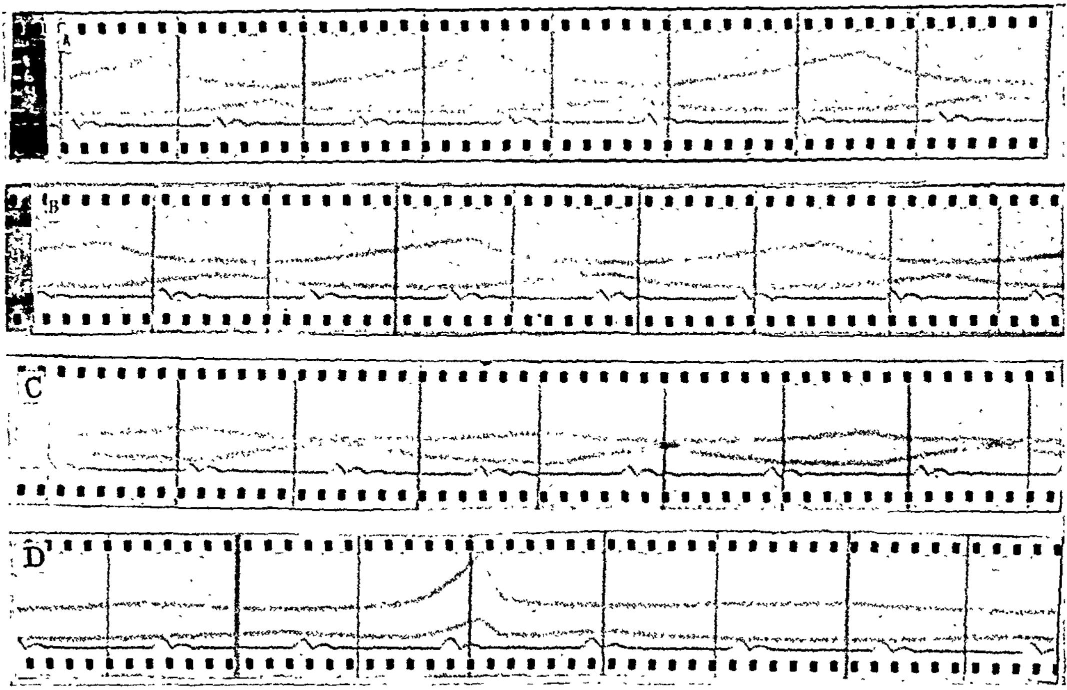

Fig. 4. Spontaneous nystagmus. A, B, and C: Experiment of 4/3/29. Internal rectus above; external rectus below. Standardization for tension of external rectus in A; for internal rectus in B. D: Experiment of 4/12/29. Retraction reflex from stimulation of nostril on a background of nystagmus. Internal rectus above; external rectus below. Retractor bulbi is free. The shortening in external rectus is probably due solely to tug from the origin of retractor, that in internal rectus chiefly to active contraction.

component averages slightly longer when stretched, in another (4/3/29 slightly shorter. The quick component is virtually unaffected. Active wink retraction in one muscle associated with probably passive pull in the antagonist leaves the nystagmus unchanged in rhythm or intensity (fig. 4 d). When nystagmus is induced calorically, the slow component is at first long, shortens as the intensity of the response increases, lengthens again as the reflex weakens. In marked contrast is the course of the quick

component, which may be constant within the limit of error of measurement throughout the entire period (fig. 5).

In two experiments an attempt was made to demonstrate nystagmus in retractor bulbi. Brisk lateral nystagmus was induced in the intact eye by irrigating the ear with cold water. With insertions of the recti and obliqui severed but the retractor untouched, irrigation induced visible minute movements of the eyeball. The eye was then enucleated but put back in the orbit. Retractor responses from stimulation of the conjunctiva still caused slight retraction of the eyeball. Nystagmus induced no perceptible movement. The eyeball was removed and the animal prepared for recording. In the internal and external recti nystagmus was vigorous. With all muscles free there appeared to be weak contraction of the retractor—so weak that we were unable to record it. The recti were then fixed

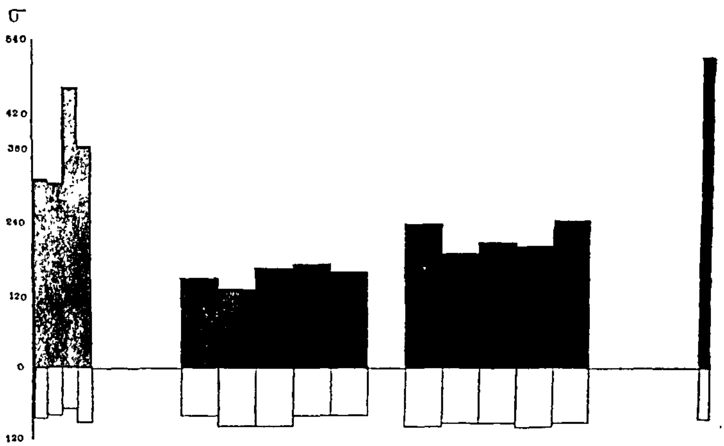

Fig. 5. Nystagmus from irrigation of ipsilateral ear with cold water. Experiment of 4/10/29. Duration of slow component plotted above the base line, that of quick component below it. Each upright represents a single beat. Thickness of uprights is proportional to change in tension. Gaps indicate periods in which the nystagmus is not plotted.

isometrically. The retractor became completely quiescent, nor could we induce in it any trace of response to irrigation while the recti were prevented from shortening.

Discussion. Reciprocal innervation was demonstrated for extra-ocular reflexes from the retina by Sherrington (1894) and for vestibular nystagmus by de Kleyn (1925). We know of no published records, however, obtained synchronously from antagonistic extraocular muscles under conditions sufficiently free from inertia to permit detailed analysis of their changes in tension or length. In the case of muscles of the extremities such a comparison is complicated by differences in distance of insertions from the moving joint and hence in effective tension and in degree of shortening.

In the case of the recti muscles of the eye the condition is almost perfectly symmetrical in length of muscle, direction of pull, and distance of insertion from the centre of rotation. We believe the levers we have employed permit an amount of shortening which approximates that occurring in the intact organism (about one millimeter per gram or about four millimeters in a vigorous nystagmus), and hence that our recorded tensions are of the order of magnitude of those which obtain in life.

If this be true for nystagmus, it cannot be true for the reflex of retraction. In this reflex the ratio of movement to tension in life is probably far smaller than that allowed by our levers when the muscles are attached to the distal holes on the lever shanks. For this reflex, therefore, our recorded tensions are probably considerably lower than those occurring in the intact animal.

In recording the retraction reflex in the recti, it proved essential to fix the retractor bulbi to avoid an artifact due to the pull at its origin upon the recording muscles. Dusser de Barenne and de Kleyn (1928), working with rabbits, have reported active participation of retractor in lateral nystagmus. The question arises whether such a response of the muscle exists in the cat, and, if so, whether it has complicated our records of nystagmus.

In view of the quiescence of retractor when the recti are prevented from shortening, we believe that in the cat the retractor does not share in nystagmus. We suspect the difference between our results and those of Dusser de Barenne and de Kleyn are due to a difference in reflex pattern between cat and rabbit. A difference in innervation of the retractor in the two species have been reported by Krause (1884) who states that in the rabbit the muscle is supplied by the oculo motor, in the cat by the abducens. It is of interest that Dusser de Barenne and de Kleyn report that section of the oculo motor abolishes nystagmus in this muscle but leaves its retractor function unimpaired. In the cat, some of our strongest retractor responses were obtained after section of the third nerve. G. S. Hopkins (1916) gives an interesting review of the conflicting literature on the innervation of this muscle. On the basis of dissections under a binocular microscope, he concludes that the innervation is solely from the abducens in cat, rabbit, and all other species he had examined. Clearly, it is unsafe to draw conclusions concerning the rôle of retractor in nystagmus in one species from data obtained upon another.

The origin of the quick component of nystagmus is still obscure. Bartels' theory, which invokes proprioceptors in the reacting extraocular muscles, has received scant credence. Yet the evidence against it is inconclusive. Bartels (1914) injected cocaine into the orbit in rabbits, and observed that both components were paralyzed. de Kleyn (1922) took advantage of the demonstration by Liljestrand and Magnus (1919) that small injections of novocaine abolish the tone of skeletal muscles due to their

own proprioceptors without impairing their exogenous reflexes. After enucleation, he injected the external rectus with varying doses of novocaine and found that with any dose which impaired nystagmus the slow component faded out pari passu with the quick. His conclusion that the proprioceptors must have been paralyzed before the disappearance of nystagmus rests upon no evidence of failure of proprioceptors in extraocular muscles, but solely upon the assumption of a vulnerability similar to that in the limb muscles injected by Liljestrand and Magnus. The work of Fulton and PiSuñer (1928), Denny-Brown (1928) and Matthews (1931) points to muscle or tendon spindles as responsible for the stretch reflex, which is presumably the factor eliminated by novocaine. In the rabbit, the extraocular muscles contain no spindles. Here the characteristic afferent endings are the terminaisons en grape (M. Hines, 1931). There is no direct evidence for the assumption that these endings or their afferents are more susceptible to novocaine than the motor nerves or endplates. Furthermore, the abrupt relaxations typical of the retraction reflex are strongly suggestive of the autogenous reflex inhibition implied in Bartels' theory. Further evidence would appear desirable.

Were the receptors of extraocular muscles the source of the quick component, the mechanism might be either a stretch reflex in the previously relaxing muscle which would inhibit the contraction in the antagonist, or a lengthening reaction in the previously contracting muscle which would excite a shortening reaction in the antagonist, or some combination of the two.

Our twenty-three experiments on stretch cover the entire range of magnitude and rate occurring in nystagmus. In only four did the tensions exceed the controls made after cutting the motor nerve. All four were in animals with reflex paths from neck and labyrinths intact. In all four the lowering of the head involved movement of the neck. In none could a jerk response be elicited by tapping the head holder. The difference in tension from that observed in the controls never exceeded that shown in figure 1. Therefore we regard them as instances of low grade contraction from exogenous reflexes (probably labyrinthine) and conclude that we have been unable to demonstrate the stretch reflex in extraocular muscles. In this we confirm the earlier results of P. Hoffmann (1929). Furthermore, the abrupt application or release of stretch during the course of a nystagmus has little if any effect upon the rhythm. Such changes as were observed do not exceed the limits of spontaneous variation, are inconstant in direction, and are far more pronounced in the slow than in the quick component (table 1). Active contraction, even when far exceeding the range occurring in lateral deflections of the eye, has no perceptible effect upon the rhythm of an intercurrent horizontal nystagmus. In figure 4 d a retraction reflex occurs upon this background. In this experiment there was no fixa-

tion of retractor bulbi. Comparison with experiments where this factor was controlled justifies, in our opinion, the conclusion that internal rectus (above) contracts powerfully, external rectus (below) passively transmits the pull from retractor bulbi. Neither event has a detectable influence upon the pattern or rhythm of the nystagmus. Hence we conclude in agreement with de Kleyn (1922) that it is highly improbable that either contraction or stretch of extraocular muscles determines the quick component of nystagmus.

Another theory worth considering suggests a labyrinthine origin for the quick as well as for the slow component. de Kleyn and Versteegh (1927) have induced post-rotation nystagmus in a rabbit in which one labyrinth was completely extirpated, the saccular and cochlear nerves cut and the ampulla of the posterior canal destroyed in the other. de Kleyn and Magnus (1921) have found nystagmus in the guinea pig after destruction of the otolith apparatus by centrifugalization. If the quick component be activated in the labyrinth, the most probable receptors would appear to be those of the ampulla which are responsible for the slow component also. Were this the case, any stimulation affecting the slow component would presumably affect the quick component also and to a commensurate degree. Hence the duration ratio of $\frac{\text{slow}}{\text{quick}}$ components should approximate a constant value, or, if deviations occur, they should bear a systematic relation to the intensity of the response. In a single experiment we find ratios ranging from 1.6 to 7.2. The low ratios include some of the lowest and some of the highest frequencies. We have found no relation between the relative duration of the two components and the intensity of the response. Figure 5 plots a response to irrigation of the ipsilateral ear with cold water. At the onset, the slow component is long, 460 σ to 300 σ. During the height of the response it drops to less than half these values. As the reaction subsides it stretches to durations of half a second. Throughout the entire period the quick component remains constant within the limit of error of measurement. In our opinion these data would be extremely difficult to reconcile with the assumption of an origin of the quick component from the receptors of the semicircular canals.

SUMMARY

Working on decerebrate cats we have been unable to demonstrate the stretch reflex in extraocular muscles.

The retraction reflex associated with the protective wink employs all four heads of retractor bulbi and often internal or external rectus in active contraction. The pull from these muscles is passively transmitted by the remaining recti through their close anatomical association at the ring of origin in the depth of the orbit. The reaction is one of rapid recruitment

to a tension which may exceed 40 grams but is frequently between 10 and 20 grams. The response is usually cut short before or shortly after attaining plateau level by relaxation which frequently approximates the speed of that which terminates a neuromyal tetanus.

In nystagmus the change in tension per beat rarely exceeds four grams and is frequently less than 1 gram. When the frequency is low, contraction recruits at an almost constant rate; relaxation in the antagonistic muscle begins more abruptly, ends more gradually. In rapid nystagmus contraction approximates a d'emblée response.

The rhythm of nystagmus is not disturbed by sudden application or release of passive stretch nor by the interpolation of a retraction reflex which contracts a muscle engaged in the nystagmus. These findings are opposed to Bartels' theory of origin of the quick component in proprioceptors of the extraocular muscles.

The duration ratio of $\frac{\text{slow}}{\text{quick}}$ component may vary from 1.6 to 7.2 in a single experiment. During the response to caloric stimulation the slow component is at first long, is reduced in duration by over 50 per cent during the height of the reaction, elongates again as it weakens. The duration of the quick component may be constant throughout the entire period of stimulation. These relations would be difficult to reconcile with a theory of origin of the quick component in the semicircular canals.

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PULSE WAVE VELOCITIES IN NINETY SUBJECTS[1]

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In reviewing the literature on pulse wave velocity we found reports based upon simultaneous subclavian-radial, carotid-radial, cardiogram-radial and brachial-radial tracings. None, however, presented the velocities in the same group of subjects for carotid-brachial-radial and subclavian-brachial radial. Since many reports have been based upon carotid-radial tracings and as this method is indirect, it being necessary to subtract the innominate-carotid from the innominate-radial distance, it seemed desirable to compare the velocities thus determined with the subclavian-radial velocities in the same group of subjects.

In view of the finding of Bazett and Dreyer (1) that the velocity in small arteries is much greater than in large, it seemed very likely that the velocities found for subclavian radial would differ from those for carotid-radial. At the same time we have made a number of additional observations, not all new, such as comparison of pulse wave velocity in men and women; confirmation of Bazett and Dreyer's finding; correlation of velocity with arterial blood pressure, systolic and diastolic, with heart rate and with other factors; effect of the respiratory arrhythmia and of auricular premature beats. We also have data upon family history of hypertension, height, weight and age. The study is of particular interest, however, in view of the recent report of Fulton and McSwiney (2) that there is no correlation between pulse wave velocity and arterial blood pressure in different individuals, since we find a significant correlation among women but not among men.

METHOD. The optical method was employed throughout. The source of light was a projection lantern. In order to obtain a sufficiently narrow band of light, a card with a vertical slit was placed before the lantern in the manner described by Wiggers (3). A Frank segment capsule was used for the subclavian or carotid artery, and for the brachial and radial small polygraph tambours. The latter were more sensitive than the Frank capsule and proved more satisfactory for the smaller arteries. These

[1] Experimental work done in the Department of Physiology, Tulane University Medical School.

capsules or tambours were placed very carefully in a horizontal line, somewhat less than a meter from the slit of the string galvanometer camera. To one spoke of the wheel of the timer an extremely light strip of bamboo was glued. This strip cut through the three beams of reflected light from the tambour mirrors in such a manner that, as the wheel rotated, the three shadows thus formed fell upon the camera slit simultaneously. Parallax was thus avoided. The timer was checked for every two or three subjects against a Jacquet chronometer of known accuracy. The speed of the film was nearly always between the limits of 60 and 70 mm. per second, quite sufficient for accurate measurement.

To insure equal transmission times of the pressure changes from the receiving to the recording parts of the apparatus, equal lengths of small, fairly rigid rubber tubing were used. Tests showed no detectable difference in transmission times. The receiving parts, placed over the arteries, were of two types. For the carotid or subclavian, and for the brachial, shallow cups or capsules, fitted with outlet tubes, were cut from large corks. The open face of the one over the brachial was covered by a rubber membrane from which the center was cut. This capsule, when lightly taped over the artery in the cubital fossa, was rendered air-tight by the use of glycerin and undue pressure was thus avoided. Pressure somewhat greater than that used was found by test not to delay the pulse wave at this point. The most satisfactory method of recording the radial pulse was by means of a thin-walled, flexible bulb of a medicine dropper connected to the rubber tubing by a glass tube conveniently bent. This receiver was also taped in position.

In recording with the subject sitting at ease, the radial and brachial receivers were fixed in position and the receiving capsule for subclavian or carotid artery held firmly over the artery by hand. The simultaneous tracings of subclavian or carotid, brachial and radial made possible simultaneous determination of subclavian-brachial (or cartoid-brachial) and brachial-radial velocities.

To determine the length of the artery, the procedure was that of Bramwell, Hill and McSwiney (4), excepting that the distal measurement extended to the point on the radial over which the receiving bulb was placed.

Records were obtained from 90 subjects and in 14 of these tracings were made on two separate occasions. Forty-two of the subjects were women; 48, men. In 49 subjects both subclavian and carotid records were secured; in 40, subclavian only; in 1, carotid only. In a few instances, the brachial record was too poor for accurate measurement.

From each subject was also obtained a statement of age, height, weight, and any known family history of hypertension or cardiac disease. The arterial blood pressure, systolic and diastolic, was estimated by the auscultatory method both before and after recording the pulse waves. The heart

TABLE 1

Subject Number	Sex	Age	Blood Pressure	Heart Rate (Subclavian)	S-R Velocity	Average S-R Velocity	Heart Rate (Carotid)	C-R Velocity
1	M.	40	110-76	59	7.40	6.90		
			118-82	75	8.55	10.30		
6	M.	21	138-90	83	7.45	14.50	84	8.30
			140-90	100	7.25	12.10		
11	M.	22	112-77	91	7.00	11.10		
			115-81	80	6.30	12.80		
12	F.	26	102-73	92	6.30	6.80		
			102-65	86	6.40	8.40		
14	M.	31	120-66	59	7.00	11.80		
			113-75	79	8.00	10.50	84	8.75
17	M.	27	107-78	76	8.25	10.30		
			106-70	90	8.20	12.50		
19	M.	23	112-78	65	6.40	9.00		
			118-78	57	6.70			
21	F.	29	110-84*	71	8.90	11.20		
			112-85	72	11.00	11.90		
25	M.	37	138-86	121	8.25	9.90		
			115-79	92	7.60	8.40		
27	F.	24	110-76*	83	7.65	9.90		
			106-74	78	7.70	9.90	76	8.90
31	M.	31	112-82	93	8.15	13.90		
			113-79	92	8.70	12.30		
33	F.	22	120-90	58	8.60	10.90		
			111-83	73	8.00	9.75		
49	F.	20	113-72	122	7.50	9.30	121	8.40
			104-70	89	6.80	8.40	100	7.15
80	F.	24	111-75	113	8.55	10.00		
			108-76	91	6.15	7.10	92	6.80
2	F.	33	102-74	77	6.70	9.05		
3	M.	22	96-68	59	7.30	12.50		
4	M.	22	112-78	75	6.50	11.80		
5	M.	23	125-82*	95	7.40	9.85		
7	M.	25	112-74	99	6.85	10.30		
8	M.	22	105-75	77	7.10	9.95		
9	F.	23	84-64	66	6.00	8.05		
10	F.	32	106-76*	90	7.20	8.80		
13	F.	25	105-78	91	7.90	11.20		
15	M.	21	107-80	70	6.00	6.45		
16	M.	20	104-76	76	7.05	10.50		
18	M.	26	109-78	80	7.30	12.60		
20	F.	22	110-84	70	8.60	8.50		
22	M.	21	111-65	77	7.05	10.90		
23	M.	22	107-82	76	7.45	11.10		
24	M.	27	110-72	80	6.40	11.00		
37	M.	23	124-80	70	8.90	12.90		
38	M.	21	126-84	73	7.80	14.70		
39	M.	20	104-70	88	7.60	12.80		
40	M.	24	123-78*	71	7.95	13.90		
41	F.	22	106-75	70	7.20	9.00		
42	F.	23	96-63	86	6.75	9.80	78	7.40
43	F.	22	123-82	80	7.20	11.05	76	7.40
44	F.	22	96-57	62	5.45	10.55	69	6.20
45	F.	30	101-73	75	8.30	11.30	77	9.10
46	F.	21	112-77	91	7.30	8.85	92	8.50
47	M.	24	114-85	78	8.00		83	9.20
48	F.	20	108-74*	86	6.85	9.40	86	7.30
50	F.	19	118-78*	101	7.90	12.70	107	10.20
51	M.	25	117-72	75	7.70	9.70	82	10.30
52	F.	17	105-73	82	7.50	16.30	87	7.60
53	M.	20	115-82	63	7.50	12.60	66	8.50
54	F.	31	128-79*	94	8.00	10.75	97	9.30
55	F.	36	128-82*	64	8.70	11.80		
56	M.	20	120-74*	48	6.70	10.10	59	7.15
57	M.	21	103-75	65	7.35		70	7.75
58	M.	44	110-65	68	7.30	9.40	70	7.40
59	F.	21	103-63	63	6.40	9.20	69	6.95
60	F.	22	112-78*	74	5.80		80	7.25
61	M.	27	117-86	76	7.70	14.55	75	8.55
62	M.	22	140-86*	64	6.95	10.25	64	8.70
63	M.	24	117-84	79	8.45	11.60	92	9.35
64	M.	22	118-85			11.80	68	7.95
65	M.	22	108-85	73	7.25	7.30		
66	F.	18	112-74*	84	7.50	10.70	85	8.20
67	F.	19	96-64	86	6.15	9.75	91	6.50
68	F.	21	104-72	98	7.60	11.20	94	8.80
69	F.	17	102-68	84	7.20	8.80	87	7.60
70	F.	19	128-88*	89	7.90	11.75	92	8.50
71	F.	22	106-73	88	6.90	9.20	91	7.60
72	M.	24	122-84	44	7.20	13.70	50	8.20
73	F.	19	122-78	88	8.40	11.35	94	9.40
74	F.	21	106-72	93	7.10	11.40	86	7.80
75	F.	25	128-76	82	6.70	9.30	90	6.85
76	F.	23	106-70	68	6.45	8.80		
77	F.	22	138-94	91	7.60	10.60	92	8.05
78	F.	20	116-76	72	6.95	11.15	78	7.80
79	F.	24	108-74	78	5.85	8.20	88	6.45
81	M.	20	114-80	80	7.20	10.60	83	7.85
82	M.	21	120-86	85	6.45	11.65	94	7.30

TABLE 1—*Concluded*

SUBJECT NUMBER	SEX	AGE	BLOOD PRESSURE	HEART RATE (SUBCLAVIAN)	S-R VELOCITY	AVERAGE B-R VELOCITY	HEART RATE (CAROTID)	C-R VELOCITY	SUBJECT NUMBER	SEX	AGE	BLOOD PRESSURE	HEART RATE (SUBCLAVIAN)	S-R VELOCITY	AVERAGE B-R VELOCITY	HEART RATE (CAROTID)	C-R VELOCITY
26	M.	35	114–82	83	8.50	10.10			83	F.	21	103–73*	79	7.50	10.95	82	7.85
28	M.	26	150–85*	86	8.00	9.75			84	M.	22	116–80	89	8.85		96	10.15
29	M.	21	112–75	78	7.05	10.60			85	M.	21	120–77	60	6.60	8.65	68	7.00
30	M.	23	118–75	73	6.30	10.90			86	F.	23	109–64	66	5.75	5.70	74	5.80
32	M.	29	111–79	92	8.30	12.30			87	M.	19	140–88	72	6.50	12.15	72	7.75
34	F.	20	98–58	72	5.55	6.90			88	M.	20	130–80	66	6.35		67	7.30
35	F.	18	106–65	71	6.40	9.90			89	M.	22	122–68	62	7.00	13.70	62	8.20
36	F.	28	107–78	91	9.20	11.40			90	M.	21	120–75	56	7.00	12.20	64	8.70

* Indicates family history of hypertension.

rate was determined directly from that portion of the tracing on which the other measurements were made.

Time intervals between pulse waves were measured by reference to the most convenient time line. An accurate millimeter ruler and hand lens were used for this purpose. To fix the beginning of the up-stroke of each wave, a mark was first made with a fine-pointed needle. Generally the time intervals used in calculation are averages of individual measurements of not fewer than ten different beats. Many of the tracings were measured independently by each of us and the agreement was remarkably close.

Results and discussion. 1. *Pulse wave velocity range and average.* The carotid-radial velocity averages 8.007 ± 0.098 meters per second in 49 subjects, with a range of from 5.80 to 10.30. This is a considerably higher velocity than that reported by Bramwell, Hill and McSwiney (4) for comparable ages, namely, 6.45 meters per second. Some of the reasons for the higher velocity in our cases are apparent. In the experiments of the above authors, the radial pulse was recorded from a cuff, 8 cm. wide, around the forearm. Thus the 8 cm. had to be subtracted from the total length of artery for their calculation. Since the highest velocities are in the smallest arteries, their procedure will necessarily give a velocity lower than that obtaining for the full carotid-radial distance. Calculation from our own data shows, however, that this factor probably does not account for more than about 25 per cent of the discrepancy. Thus, if this factor were allowed for, our mean velocity would still be about 7.65 meters per second. Another factor is perhaps the more complete relaxation of Bramwell, Hill and McSwiney's subjects. It is also not impossible that the method employed by them for recording the radial pulsation produced a delay as compared with that used by us. If so, this would tend to make their velocities too low.

The average subclavian-radial velocity on the same group of 49 subjects was 7.163 ± 0.071 meters per second, with a range of from 5.45 to 8.85. This average is 0.844 ± 0.121 meter per second slower than the C-R (carotid-radial) velocity already given. In view of the magnitude of the difference and of its probable error, there can be no doubt that any large group of subjects would present a considerable difference in C-R and S-R (subclavian-radial) velocities. Individually there was a fairly wide range in this difference, i.e., from 0.05 to 2.60 meters, but in no case was the S-R velocity greater than the C-R.

The reason for the difference in these velocities is not difficult to understand. The chief factor was undoubtedly the size of the artery. In calculating the C-R velocity, the arterial length used was determined by subtracting the innominate-carotid distance from the innominate-radial. Since the common carotid is large, the effect is equivalent to subtracting a part of this length of large artery from the subclavian-radial distance. The difference in the two velocities, therefore, is added confirmation of Bazett and Dreyer's observation that the pulse wave is propagated more rapidly in small than in large arteries.

Assuming that the pulse wave velocity in the subclavian-axillary is, on the average, equal to that in the common carotid, we may make an indirect calculation of the velocity in the latter. The velocity calculated is 3.15 meters per second. The mean diastolic blood pressure was about 76 mm. of mercury. If we allow about 16 mm. for the difference in hydrostatic pressure between the carotid and brachial in the sitting position, we may estimate the mean carotid diastolic pressure at about 60 mm. Hg. The pulse wave velocity in isolated carotid arteries from the younger individuals in Bramwell, Downing and Hill's (5) experiments was about 5 meters per second at this pressure (their fig. 3). Bramwell, McDowall and McSwiney (6) reported an average velocity of approximately 4.5 meters per second at a pressure of 60 mm. in the artery *in situ*; but in their experiments a smaller artery, apparently the brachial, was used. In an earlier paper, at a pressure of 57 mm. in the isolated carotid artery of a young man, Bramwell and Hill (7) found a velocity of 3.45 meters. Bazett and Dreyer estimated the velocity from heart to carotid at about 3 to 4 meters at a diastolic pressure of 70 to 75 mm. Therefore the velocity of 3.15 which we calculate for the common carotid is in fair agreement with the findings of other authors, although the method of indirect calculation involves an assumption which may not be true.

There was a group of only 43 subjects in whom both carotid-brachial and subclavian-brachial velocities are available. In these, in contrast to an average C-R pulse wave velocity in the same group of 7.98 meters per second, the mean C-B (carotid-brachial) velocity is 6.83. This latter velocity is lower, of course, because of the average larger size of the vessel.

In the same way the average subclavian-brachial velocity is but 6.01 meters per second in the same 43 subjects in comparison with an average S-R velocity of 7.15. The difference between S-R and C-R is 0.83 meter, and between S-B and C-B, 0.82.

The velocity in the radial artery, in agreement with the finding of Bazett and Dreyer, is considerably higher than that in the other arteries. Our mean brachial-radial velocity, from 98 determinations, is 10.60 ± 0.131 meters per second, with individual variations from 5.70 to 16.30. Bazett and Dreyer state that the average velocity from elbow to wrist is 8.5 meters per second, although an average B-R velocity of the ten normal cases given in their tables is exactly 10.00 meters. This is very close to our average.

According to Moen's (8) formula, the factors, aside from pressure, which may influence the velocity are the elasticity and thickness of the vessel wall and the diameter of its lumen. As Bazett and Dreyer suggest, it is these factors, particularly the diameter of the vessel, which account for the higher velocity in the radial artery. As Sands (9) suggests, no doubt various influences, among which the nervous is probably the more important, may very considerably modify the pulse wave velocity, particularly in the radial.

In recapitulation, our evidence shows that the velocity of the pulse wave in general increases in the following vessels in the order named: subclavian-axillary and carotid, brachial, radial.

2. *Diastolic pressure and pulse wave velocity.* There is every reason to believe that the factor of primary importance in determining pulse wave velocity in a given vessel is the diastolic blood pressure (6), (7). With this in mind, we have compared the velocities, not only from the subclavian or carotid to radial, but also from the subclavian or carotid to brachial and from the brachial to radial, with the diastolic pressures. If the table be examined, it will be seen that there is a general correspondence between the two, but that there are numerous individual exceptions. To a large extent, no doubt, the exceptions are to be attributed to other factors, such as elasticity of the arterial wall or average caliber of the lumen. In calculating the coefficients of correlation, we have included in each case the two readings from each subject upon whom the determination was repeated. Such inclusion, while not an orthodox statistical procedure, affects the results hardly at all. The average or mean S-R velocity in 102 determinations (including 13 repetitions) is 7.33 ± 0.06 meters per second, with a mean diastolic pressure of 76.5 ± 0.47 mm. Hg. The standard deviations were respectively 0.8935 ± 0.0423 and 7.020 ± 0.337. The correlation coefficient was 0.496 ± 0.050. Since the probable error is only about a tenth of the coefficient, the correlation is certainly significant. A

coefficient of correlation of this magnitude means that there is about a 67 per cent association between the pressure and the velocity (10).

In comparison with these figures, in 96 determinations where the readings were available, the average subclavian-brachial velocity was 6.26 ± 0.066 meters per second, with a mean diastolic pressure of 76.77 ± 0.515 mm. Hg, and a coefficient of correlation of 0.384 ± 0.059. Here the association is less close, but it is, nevertheless, significant.

Brachial-radial velocities were available from 98 determinations. Here the mean velocity was distinctly higher, 10.60 ± 0.131 meters per second, while the mean diastolic pressure was 76.99 ± 0.515 mm. Hg. The coefficient of correlation was 0.328 ± 0.061, again significant, but with a percentage of association of only about 61. It is to be expected that other factors than pressure, such as vessel caliber, will be a more important influence in small than in large arteries. A further calculation shows that there is no significant correlation between B-R and S-B velocities. This would appear to mean that many cases which showed a correspondence between diastolic pressure and the one velocity gave none for the other.

Turning to our carotid measurements on 49 subjects, the mean C-R velocity was 8.025 ± 0.103 meters (by indirect calculation, compared with the direct given above); the mean diastolic pressure 76.174 ± 0.759 mm. Hg; and the correlation coefficient 0.395 ± 0.083. In comparison with these figures, the S-R velocity in the same 49 subjects was 7.199 ± 0.077, and the coefficient of correlation with diastolic pressure, 0.336 ± 0.086. It appears, therefore, that C-R and S-R pulse wave velocities correlate about equally well with diastolic pressure.

3. *Systolic blood pressure and pulse wave velocity.* The coefficient of correlation for these was 0.246 ± 0.065. This does not mean that the velocity is in any way directly influenced by systolic pressure, but, as we interpret it, that in general a high systolic means a relatively high diastolic pressure and vice versa.

There was no significant correlation between pulse pressure and pulse wave velocity, the calculated coefficient being −0.100 ±0.067.

4. *Sex differences.* The average heart rate in 55 determinations on 47 male subjects was 77.09 ± 1.27 beats per minute, and the diastolic pressure, 79.32 ± 0.555 mm. Hg. In 47 determinations on 42 female subjects the figures were 81.91 ± 1.26 and 74.21 ± 0.763 respectively. Along with these differences in heart rate and diastolic pressure, there were corresponding differences in mean S-R pulse wave velocity; the figures for men and women being 7.423 ± 0.067 and 7.239 ± 0.097 respectively, a percentage difference of 2.5. The B-R velocities were distinctly higher in the men, 11.26 ± 0.191 against 9.947 ± 0.175 for the women, a percentage difference

of 13.2. These differences are probably to be explained on the basis of the difference in diastolic pressures in the two groups.

When we separate the sexes and correlate the pulse wave velocities with the diastolic blood pressures, unexpected differences appear. In the 47 determinations on women the correlation (S-R and diastolic pressure) was 0.662 ± 0.0565, a percentage of association of nearly 74. In the 55 determinations on men the correlation was 0.0934 ± 0.091. Among the women, therefore, the correlation is fairly close, while in men it is absent. The same difference emerges when we correlate carotid-radial velocities and diastolic pressures; for 29 women, 0.529 ± 0.093; for 20 men, 0.146 ± 0.156. The similar correlations using brachial-radial velocities show, for 47 determinations on women, 0.326 ± 0.090 and for 50 determinations on men, 0.0813 ± 0.0935.

The discovery of this difference led us to wonder whether accurate estimation of diastolic pressure is more difficult in the more muscular arms of men than of women. That the contrast is not due to such an error is clearly shown, however, by a separate correlation in the two sexes between systolic pressures and velocities. The same sex difference again appears in quite unambiguous fashion, the coefficients being 0.550 ± 0.0702 and 0.057 ± 0.0895 respectively for women and men. Dr. James A. Bradley suggested to us that a greater variation in arterial caliber in men than in women, due to greater differences in muscular development in the former, may explain this remarkable and, at least statistically, significant sex difference. In this connection it is of interest to note that Fulton and McSwiney (2) report no correlation between blood pressure and pulse wave velocity in different subjects. Although the authors do not so state, it may be that their subjects were chiefly male.

The correlation between heart rate and S-R velocity in 55 determinations on 47 men is 0.329 ± 0.081; in 47 determinations on 42 women, 0.172 ± 0.0975. In neither case is the percentage association great, although there appears to be a slight relation between rate and velocity, probably dependent on the fact there tends to be, *ceteris paribus*, an increase in pressure with a rise in rate. The difference here shown between men and women is probably not statistically significant.

5. *Heart rate and diastolic blood pressure.* Correlating the heart rate and diastolic pressure in 103 determinations on 89 subjects, we obtain a coefficient of 0.0915 ± 0.066, not a statistically significant relation. However, when the heart rates of the two sexes are adjusted to a common basis and the correlation then made, the coefficient becomes 0.167 ± 0.065, that is, nearly three times its probable error. We may, therefore, say that, other things being equal, there is probably some relation between the two, but other factors preclude a significant correlation.

6. *Heart rate and pulse wave velocity.* Here again the correlation

coefficient, 0.250 ± 0.066, while higher than that relating rate and diastolic pressure, is not high. When adjustment is made for the sex differences in heart rate the coefficient rises slightly to 0.277 ± 0.062.

7. *Variations depending upon physiological state of the individual.* In case of several of the subjects upon whom duplicate determinations were made at different times, striking differences in pulse wave velocity were recorded. For convenience these have all been placed at the head of the accompanying table. It will be observed that there is a change in the velocities when both heart rate and diastolic pressure vary considerably. This observation serves to emphasize the necessity of complete relaxation of the subject if what might be called the basal pulse wave velocity is to be determined. On one occasion, not shown in the table, we attempted to determine the change in velocity in one arm while the opposite arm was immersed in ice water. The increase in S-R velocity was from 6.21 to 7.05 meters, but it is impossible to tell whether it followed a rise in pressure due to vasoconstriction produced by cold or by discomfort. Observation demonstrated a rise in diastolic pressure with immersion of the arm. Sands' (9) experiments in which dry heat applied to the arm was found to decrease the velocity are less open to this criticism.

Vigorous exercise, tried on one occasion, increased the carotid-radial velocity from an average of 7.65 to 8.74 meters per second.

Frequent auricular premature beats, observed in one case, produced a change in subclavian-radial velocity which was hardly greater than the probable error in the measurements. For the beats terminating the longer cycles following the premature beats the average velocity was 6.04 meters; for beats terminating cycles of average length, the velocity was 6.16 meters; and the pulse wave of the premature beats travelled 6.36 meters per second. While it may be questioned whether a real increase is demonstrated, as would be expected on theory, we are confident that the velocity of the premature waves is not less than that of the normal waves as maintained by Laubry, Mougeot and Giroux (11), (12).

Partial confirmation of the above finding is derived from a study of a case of sinus arrhythmia and bradycardia. Here the effect was unquestionable. During the sinus arrhythmia the average subclavian-radial velocity for seven beats terminating cycles of 1.2 seconds was 6.80; for seven beats ending cycles of 0.98 second the average velocity was 7.56; and for 17 beats following cycles of intermediate length, 7.20 meters. These changes are in agreement with those reported by Hickson and McSwiney (13).

Holding the breath to the breaking point was another influence causing a rise in the velocity (S-R), from 8.22 to 9.67 meters per second.

8. No significant relationship was found between pulse wave velocity and family history of hypertension.

It is important to note that in all correlations the exceptionally high

S-R velocity of 11 meters found on one occasion in subject number 21 was omitted.

SUMMARY

The average carotid-radial pulse wave velocity in 49 subjects was 8.007 meters per second; the average subclavian-radial, in 102 determinations on 89 subjects, 7.33 meters. The average velocity from carotid to brachial was 6.83, and from subclavian to brachial, 6.26. From brachial to radial it was 10.60. The velocity in the carotid artery is indirectly calculated to have been 3.15 meters per second.

There is a slight positive correlation between pulse wave velocity and heart rate.

The correlation between pulse wave velocity and systolic or diastolic blood pressure is good in women. In men both these correlations fail.

In a single case the pulse wave of auricular premature beats was found to travel, not more slowly, but if anything more rapidly than the other waves.

No significant difference was found between the groups who did and who did not report family history of hypertension.

The physiological state of the individual exerts a marked influence upon the pulse wave velocity, as detailed in the body of the paper.

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THE RESERVOIR FUNCTION OF THE SPLEEN IN FOWLS

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The discovery by Barcroft of the reservoir function of the spleen in mammals has raised the question whether this function exists in other groups of animals, and if so, whether it is subserved by the spleen. We selected the domestic fowl as a representative of a group, the birds, in which we do not believe this question has been studied.

METHODS. Single comb leghorns were used and blood obtained from a needle stab into a vein in the wing. Hemoglobin estimations were made on these samples by means of a Dare hemoglobinometer. The bird was put in a small coop in the dark for about an hour before the "resting" estimations were made and the samples taken with a minimum of scaring and stimulation.

Immediately after the "resting" estimations had been made asphyxia was induced by pinching the trachea from outside until the bird went limp. Then a sample from the opposite wing was taken as quickly as possible for the "asphyxiated" estimation.

Each figure quoted is the mathematical average of ten readings on the same sample. The standard deviation and mean error of these readings were computed and only those differences are quoted as significant in which the difference between the means of the two sets of readings is greater than four times the probable error of the difference.

The splenectomy operation was made as follows: Fifty milligrams of amytal per kilo were injected into the body cavity and the anesthesia was completed by ether inhalation.

A skin incision two inches long was made just ventral and caudal to the right wing. By blunt dissection through the peritoneal folds slightly to the right of the gall bladder, the spleen was exposed. After rupture of its capsule the organ was enucleated with the finger and bleeding checked by tight packing with dry cotton which was left in place for five minutes. After removal of the cotton the skin and body wall were closed as two separate layers with interrupted thread sutures.

Apart from ordinary cleanliness no aseptic precautions were found to be necessary. Three or four days after the operation the hens appeared

to be perfectly normal. The wounds healed by first intention and were almost invisible by the end of six weeks, which was the time arbitrarily allowed for complete recovery before further hemoglobin estimations were made—in one case the hen was already laying again.

The control operation was identical with that just described except that the spleen after exposure and identification was left intact.

The total operative and anesthetic mortality was a little over 60 per cent.

TABLE 1

The effect of partial asphyxia on the hemoglobin number of normal and splenectomized hens

HEN NO.	BEFORE SPLENECTOMY			AFTER SPLENECTOMY		
	Before asphyxia	After asphyxia	Difference in per cent of normal	Before asphyxia	After asphyxia	Difference in per cent of normal
4237	63	66	+5	63	63	0
4427	58	66	+14	63	63	0
B346	40	43	+8	62	62	0
D777*	32*	39	+22	68	67	−1
D766*	31*	34	+10	39*	38	−3
D794*	35*	37	+6	50	50	0
D771*	30*	34	+13	52	52	0

* Those hens with a low hemoglobin number were all laying, whereas those with a high number were not. There seems to be some correlation between hemoglobin value and the egg laying cycle.

TABLE 2

The effect of partial asphyxia on the hemoglobin number of normal and operated but non-splenectomized hens

HEN NO.	BEFORE OPERATION			AFTER OPERATION		
	Before asphyxia	After asphyxia	Difference in per cent of normal	Before asphyxia	After asphyxia	Difference in per cent of normal
4293	62	72	+16	56	63	+13
5574	54	61	+13	54	66	+22
5564	64	78	+22	63	67	+6

RESULTS. Tables 1 and 2 show first that in normal hens asphyxia is accompanied by an increase in the hemoglobin content of the blood. Besides those figured in the table we obtained similar data for some fifteen other birds. We observed no exception to this correlation though the degree varied from about 5 to 25 per cent increase.

This seems to indicate that the hen, like the mammalia, is equipped with some mechanism for mobilizing red cells in an emergency.

Consideration of the data presented in the right hand columns of table 1 shows that the splenectomy operation described completely inhibits this mechanism, while the control operation leaves it unaffected (see table 2). By analogy with work on mammalia we may presume that the inhibition is due to the removal of the spleen with its ability to expel its store of red cells into the general circulation.

We may conclude on the basis of this evidence that the reservoir function of the spleen is present in fowls as in mammals.

THE INDEPENDENCE OF SPONTANEOUS GASTRO-INTESTINAL MOTILITY AND BLOOD SUGAR LEVELS

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Considerable practical and theoretical interest is associated with the causal factors of spontaneous gastro-intestinal motility. Attempts have frequently been made to determine these factors, especially those concerned with the motility (hunger contractions) of the empty stomach. The evidence presented by Carlson (1919) indicating that gastric hunger motility may bear an inverse relation to the blood sugar level has attracted much attention.

Contradictory to this theory was the report of Templeton and Quigley (1930) that insulin hypoglycemia did not augment and intravenous injection of glucose did not inhibit spontaneous motility in the Heidenhain pouch or the pyloric pouch. A similar lack of relationship between blood sugar level and motility of the empty stomach was obtained with vitamin-B deficient dogs by Stucky, Rose and Cowgill (1928). Quigley and Lindquist (1930) found the hypoglycemia produced by phloridzin administration to be associated with a decrease instead of the anticipated increase in hunger contractions.

It was considered that under these experimental conditions the gastric motor mechanism was abnormal for Bulatao and Carlson (1924) had reported that intravenous administration of glucose to the normal dog was followed by a marked gastric inhibition, while insulin hypoglycemia was associated with gastric motor augmentation.

We have reinvestigated in detail the effect on gastro-intestinal motility of carbohydrate administration; gastric motility has received our chief emphasis, but the motility of the small intestine and colon has also been studied. The investigation included double vagotomized dogs as well as normal animals.

Twelve healthy dogs were selected for this investigation, five animals had experienced no surgical intervention, one had a colostomy, one had a cannula to the duodenum, two had a fistula into the terminal portion of the ileum and three had a double vagotomy and also a colostomy. Since our studies on gastric motility concerned factors which might modify this

motility, we usually carried out our experimental procedures when the motility was vigorous and was as continuous and uniform as possible.

Gastric inhibition can readily be produced by the manipulations incident to an intravenous injection. We endeavored by extensive training and careful routine manipulations of the animals to avoid the production of psychic disturbance throughout the entire experiment. We further emphasized physiological conditions by employing moderate amounts of physiologically isotonic solutions of the carbohydrates but we also employed larger quantities and also hypertonic solutions. In certain experiments we minimized the effect of pricking the skin by smearing the area with phenol paste. On other occasions we inserted into a leg vein a needle connected to a reservoir of 0.9 per cent saline. By gravity feed, saline was slowly passed through the needle to avoid an obstruction with clotted blood. At a suitable time the turning of a valve introduced glucose solution instead of saline into the vein. A period of about 20 minutes was utilized for the injection of 100 cc. We found it advantageous for the experimentor to leave the room at the beginning of this interval and watch the proceedings through a window. In the major portion of our experiments less elaborate precautions than those just described were employed, yet the results lead to similar conclusions. In all cases our solutions were at body temperature when administered. In each investigation the study was made repeatedly, usually under a variety of conditions so the results described as typical are believed to be uncomplicated by incidental factors. Gastro-intestinal motility was followed by the balloon method. "True" blood sugar determinations were made by the method of Somogyi (1929). Fasting periods varied from 18 to 64 hours at the beginning of the experiments.

Intravenous administration of glucose. In normal animals we determined on 71 occasions the effect on gastric motility of intravenous injections of glucose in quantities ranging from 1 to 25 grams. The conclusion appeared to be quite definite that hyperglycemia produced by these procedures was without immediate effect on spontaneous gastric motility. Three or four balloons in tandem, introduced into various parts of the stomach gave results similar to the single balloon and indicated that all parts of the stomach behaved similarly to intravenous glucose (fig. 1).

Occasionally a transient gastric inhibition was obtained just before, during, or after the injection. This inhibition was definitely associated with restlessness on the part of the animal,—it persisted for only 1 or 2 minutes (the original degree of motility returning while the hyperglycemia was very marked). An augmentation of motility associated with the injection was of approximately as frequent occurrence as this transient inhibition. Such augmentation or inhibition appears to be entirely incidental (related to psychic disturbance of the animal).

Another problem arising from this investigation concerns the late effects to be obtained from the administration of glucose. It has frequently been shown (c.f., e.g. Quigley, Hallaran and Barnes, 1932) that a period of

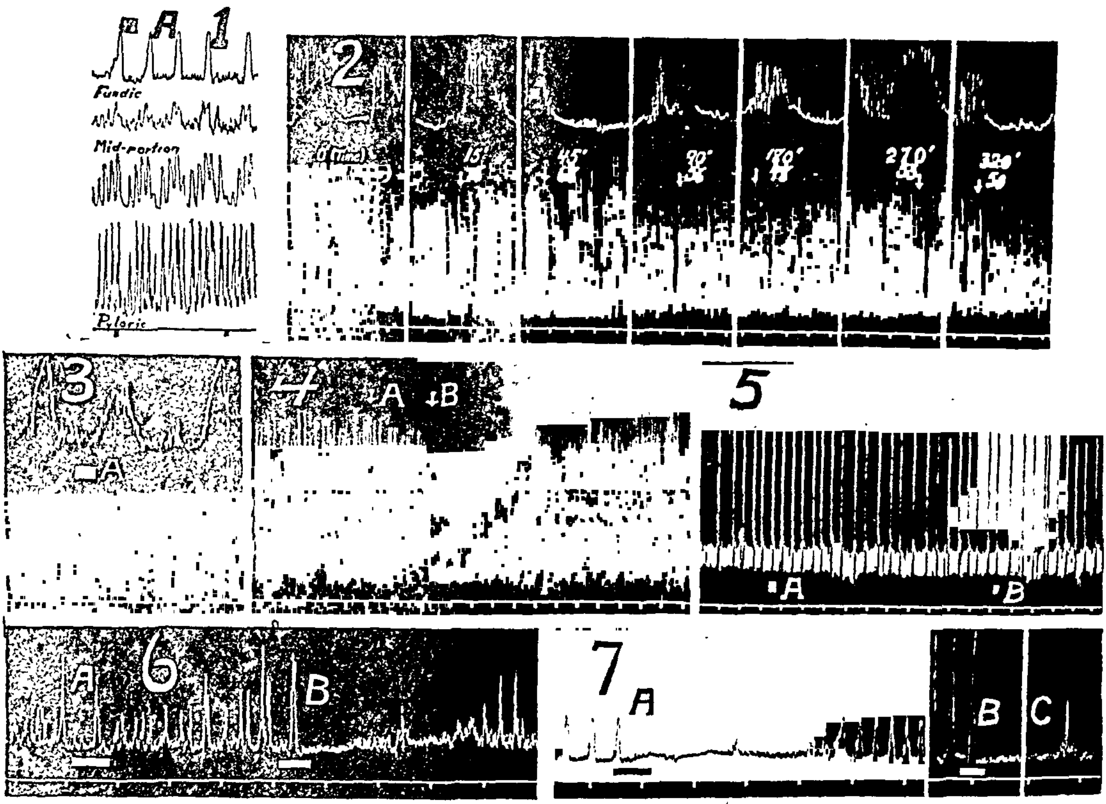

Fig. 1. Normal dog, 40 hours since fed, 4 balloons in stomach. A. Intravenous injection 10 grams glucose (30 cc.).

Fig. 2. Normal dog, 40 H.S.F., record from colon and stomach. At O' injected intravenous 0.25 gm/kgm. glucose (50 per cent).

Fig. 3. Normal dog, 34 H.S.F., record from ileum and stomach. At *A* injected intravenously 15 grams glucose (50 per cent). "True" blood sugar 10 minutes before injection; 79 mgm. per 100 cc., 60 minutes after injection 160.

Fig. 4. Normal dog, 18 H.S.F., record from stomach. A. Intravenous injection of 10 grams glucose (50 per cent). B. Intravenous injection of 0.5 cc. oxytocin.

Fig. 5. Vagotomized dog, 40 H.S.F. record from stomach. A. Intravenous injection of 50 grams glucose (50 per cent). B. Intravenous injection of 30 cc. of 0.9 per cent NaCl.

Fig. 6. Normal dog (with cannula to duodenum), 20 H.S.F., record from stomach. A. Fifty cubic centimeters 0.9 per cent NaCl into duodenum. B. Fifty cubic centimeters cane sugar (isotonic) into duodenum.

Fig. 7. Normal dog (with cannula to duodenum), 40 H.S.F., record from stomach. A. Fifty cubic centimeters glucose (5 per cent) into duodenum. B. Thirty-five minutes later, 50 cc. lactose (isotonic) into duodenum. C. Twenty-eight minutes later.

Time intervals 5 minutes.

hyperglycemia such as may be produced by administration of glucose is followed by an interval of hypoglycemia—supposedly the result of overproduction of insulin by the pancreas. It is well established that the administration of insulin to fasting animals will increase gastro-intestinal motility. The insulin produced physiologically in response to glucose administration might be expected to have a similar effect. Such a concept might explain why an individual experiences hunger 4 to 6 hours after a meal and the idea has already been employed by clinicians in prescribing the so-called "sugar breakfast" to augment hunger. Another method of attacking our problem was thus suggested since it is a procedure which modifies the blood sugar level.

We attempted to answer the questions here raised by observing the degree of gastro-intestinal motility during a period of physiological hypoglycemia, i.e., 3 to 5 hours following the administration of glucose. Results so obtained are admittedly difficult to evaluate for many extraneous factors may alter the degree of gastro-intestinal motility during an observation period of 5 hours. We were unable to make our observations more conclusive when we shortened the observation period. As the result of 63 experiments records were obtained in which an increase in gastric motility occurred during the period of hypoglycemia when the blood sugar values had fallen below normal and motility decreased as the blood sugar returned to the original level. Many records showed no such change (fig. 2) or even the reverse condition and our results only permit the conclusion that if the intravenous administration of glucose leads to a delayed increase in gastric motility the effect is slight and inconstant in its appearance.

Quigley and Solomon (1930) have shown that when hypermotility of the colon and small intestine occur during insulin hypoglycemia, complete inhibition of these portions of the gut follow the administration of glucose. We have investigated the effect of injections of glucose on *spontaneous* motility of the intestine and as the result of 17 experiments have concluded that intravenously administered glucose does not modify the *spontaneous* motility of the ileum or proximal or distal colon (figs. 2 and 3).

From the results obtained in 33 experiments we concluded that the intravenous injection of glucose produced neither inhibition nor augmentation of the spontaneous motility of the stomach or proximal or terminal colon in *double vagotomized* dogs, either immediately after the injection or later during the hypoglycemic period (fig. 5).

Administration of carbohydrate into the gastro-intestinal tract. The observation has repeatedly been made that the oral administration of food, especially carbohydrates, will readily relieve hunger and inhibit hunger contractions. Recognizing the possibility that this inhibition might be related to the production of hyperglycemia we have investigated the mechanism of gastric inhibition by carbohydrates introduced into the gut.

In an extended series of experiments we have found that the introduction of 0.9 per cent saline into the stomach in amounts even in excess of 100 cc. did not modify gastric motility in normal dogs. In 32 experiments introduction of glucose as an isotonic or hypertonic solution readily produced complete gastric inhibition which usually persisted for 13 to 50 minutes (fig. 8). This inhibition became evident within three minutes of

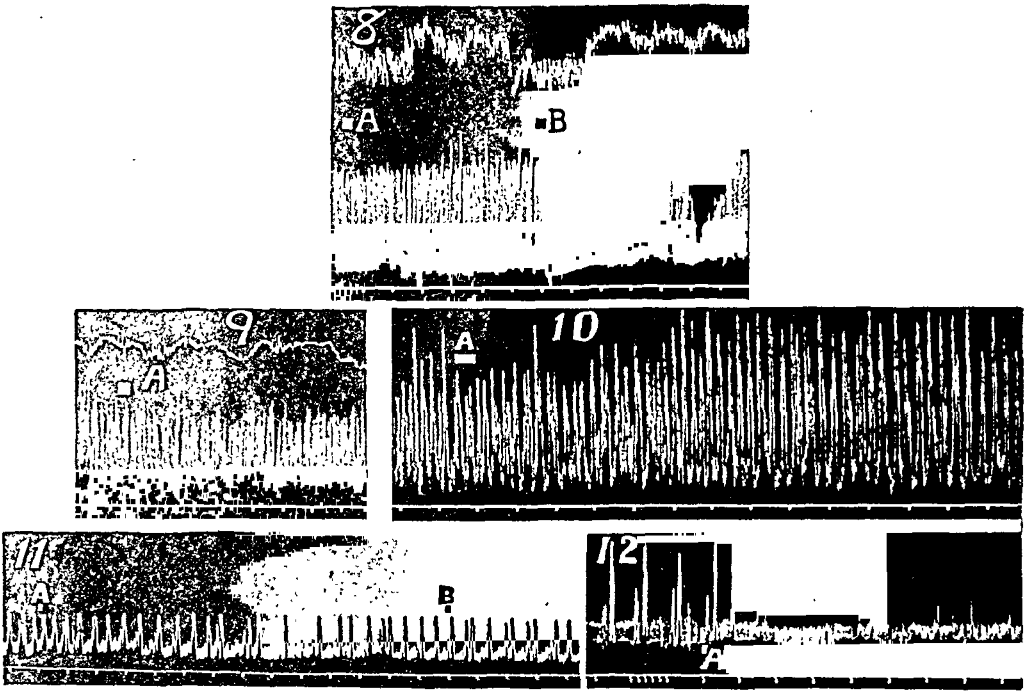

Fig. 8. Normal dog, 40 H.S.F., record from ileum and stomach. A. One hundred cubic centimeters 0.9 per cent NaCl into stomach. B. One hundred cubic centimeters 5 per cent glucose into stomach.

Fig. 9. Normal dog, 20 H.S.F., record from ileum and stomach. A. Fifteen grams glucose (50 per cent) into ileum.

Fig. 10. Normal dog, 40 H.S.F., record from stomach. A. Fifty cubic centimeters glucose (10 per cent) into colon, 20 cm. from external sphincter.

Fig. 11. Vagotomized dog, 40 H.S.F., record from stomach. A. One hundred cubic centimeters lactose (isotonic) into stomach. B. One hundred cubic centimeters 0.9 per cent NaCl into stomach.

Fig. 12. Vagotomized dog, 40 H.S.F. record from stomach. A. One hundred cubic centimeters lactose (isotonic) into stomach.

Time intervals 5 minutes.

the time administration began and was replaced by active motility at a time when glucose absorption and the attendant hyperglycemia would be reaching a maximum. It thus appeared very unlikely that the inhibition was related to the production of hyperglycemia.

Introduction of isotonic solutions of cane sugar (6 experiments) or lactose (30 experiments) into the stomach was also quickly followed by gastric inhibition. Since the hydrolysis and absorption of these disaccharides would require an appreciable interval, further evidence was provided in favor of the conclusion that gastric inhibition from carbohydrates introduced into the stomach was not the result of hyperglycemia. Typical gastric inhibition could be obtained by the introduction of carbohydrate solution into the stomach of a dog on whom the absence of effect from intravenous glucose had been demonstrated a few minutes earlier. Introduction of carbohydrate solution into the stomach did not modify motility of the terminal ileum or the colon (fig. 8).

Evidence was obtained by Quigley, Johnson and Solomon (1929) indicating that insulin gastric hypermotility was readily inhibited by glucose in the duodenum but not by glucose remaining in the stomach. In the present investigation it was possible that gastric inhibition following the introduction of carbohydrate into the stomach was related to carbohydrate in the duodenum but not to carbohydrate in the stomach. Evidence supporting this theory was obtained by passing a gold-plated cannula through the abdominal wall of a dog and attaching it to the peritoneal surface of the lower third of the duodenum. Solutions were introduced into the lumen of the duodenum by passing a long hypodermic needle along the cannula and through the duodenal wall. Introduction of physiologically isotonic glucose, lactose or cane sugar produced gastric inhibition entirely comparable to that following similar injections into the stomach (figs. 6, 7).

In vagotomized dogs the introduction of glucose (11 experiments), cane sugar (10 experiments) or lactose (8 experiments) into the stomach usually produced gastric inhibition similar to that observed in normal animals (fig. 12). Occasionally, however, gastric inhibition did not immediately follow such administration of carbohydrate to vagotomized animals (fig. 11). Retaining the theory that gastric inhibition is related to carbohydrate in the duodenum, we explained this variation in vagotomized animals as resulting from gastric retention of the carbohydrate due to the pyloric hypertonicity frequently observed in these animals.

It would appear that gastric inhibition following the introduction of carbohydrate in the upper gut is related to a reflex from the duodenum. This reflex probably is not mediated over vagus fibers since it can occur in vagotomized animals. This inhibition is probably similar to that demonstrated in the Heidenhain pouch (by Templeton and Quigley, 1930) following the introduction of glucose into the main stomach.

Absorption of glucose is believed to occur readily from the ileum and another method of investigating the effect of hyperglycemia on gastric motility was thus suggested. In 10 experiments we uniformly failed to influence gastric hunger contractions in normal dogs by the introduction of

25 to 50 cc. of glucose as a 5 to 50 per cent solution into the lower portion of the ileum (fig. 9). We likewise failed to observe appreciable changes in the blood sugar level in these experiments. Similar amounts of glucose introduced into the colon of normal or vagotomized dogs was also without effect on gastric motility (fig. 10).

We have confirmed the observation of Carlson that intravenous injection of 17 per cent saline or 0.45 gm./kgm. acetone does not modify motility of the empty stomach.

Discussion. Gastric hunger contractions can be augmented by fasting, moderate exercise, hemorrhage, exposure to cold, administration of insulin, pancreatic diabetes (Carlson and collaborators), or hepatectomy (La Barre and Destrée, 1930). Can these diversified procedures be correlated to yield a theory which is in accord with the reported observations and perhaps explain the underlying factors of gastro-intestinal motility? Luckhardt and Carlson pointed out that under the above conditions augmented gastric motility was associated with *a*, a decrease in the blood sugar concentration; *b*, a lowering of the glycogen reserve; *c*, an inability of the tissues to burn carbohydrates; *d*, acidosis of varying degree. Admittedly one or more of these four factors are encountered in each case mentioned but none are present in all the procedures augmenting gastric hunger contractions. Fasting, exposure to cold, hepatectomy or insulin administration will lower the blood sugar level, but hyperglycemia occurs in experimental diabetes and also may result from hemorrhage (Mulinos, 1928) and moderate exercise (Nolte, 1929). (Blood sugar values were not reported by Carlson in his experiments with hemorrhage and exercise.) A consideration of the problem from the standpoint of modifications in carbohydrate metabolism apparently does not supply the answer. Fasting and diabetes decrease carbohydrate metabolism; exercise, exposure to cold, insulin or intravenous glucose would augment carbohydrate metabolism. The conditions which would lead to gastric hypermotility are practically all conditions which would lower glycogen reserve. An exception is the case of insulin administration. Hypermotility begins here at a time when glycogen formation is augmented. Occurrence of hypermotility from pancreatic diabetes and also following insulin administration is especially difficult to harmonize. In depancreatized animals carbohydrate metabolism is depressed, R.Q. is decreased, metabolism of fats and proteins augmented, glycogen formation and storage in the liver decreased, formation of ketone bodies and tendency to acidosis increased. In the insulinized animal practically the opposite set of conditions exists. Perhaps we must assume in the insulinized animal a localized carbohydrate starvation for the hunger mechanism at a time when carbohydrate combustion of the body as a whole is augmented. This question of localized carbohydrate starvation deserves further investigation.

The above discussion should be considered in relation to the previously

reported observations that gastric hunger motility is not inversely related to the blood sugar level in the Heidenhain pouch, the pyloric pouch, the phlorhidzinized animal or the vitamin-B deficient dog. Combining this with our present observations that motility in the normal or vagotomized stomach is not modified by intravenous glucose and probably not by the hyperglycemia resulting from carbohydrate introduced into the gut, we feel that a satisfactory statement of the relation of carbohydrate to the control of gastric motility is still wanting.

Intravenous injections of hypertonic glucose solutions will produce marked changes in phosphate and calcium metabolism and also in osmotic relations between the blood, tissue cells and cerebrospinal fluid. It appears significant that such changes can be produced without modifying motility of the gut.

Rose, Stucky, Mendel and Cowgill (1931) have investigated the gastric atony which occurs in dogs deprived of water. They suggest that anhydremia is the cause of gastric atony. Our experiments in which marked changes in the osmotic pressure of the blood were produced by the intravenous injection of hypertonic solutions of saline or glucose fail to support this theory.

In attempting to explain the inhibition of the gastro-intestinal tract which follows the intravenous injection of pituitrin, vasopressin or oxytocin to dogs, circumstantial evidence was presented by Quigley and Barnes (1930) tending to show that the *latter part* of the inhibition period might be dependent on an elevation of the blood sugar level. Although it theoretically is not impossible that this may still be a factor in the case of dogs with insulin hypoglycemia, it certainly is not so in the normal dog. In the normal animal, not only does intravenous glucose fail to produce inhibition, but the administration of pituitrin preparations shortly after the glucose is immediately followed by the typical pituitrin inhibition (fig. 4). A similar situation holds for adrenalin: blood sugar elevation was considered as a possible factor in the inhibition of gastric motility following adrenalin administration (Quigley, Johnston and Solomon, 1929). However, in this case further evidence minimizing the importance of the blood sugar level is available in the observation of Wilder and Schultz (1930) that the blood sugar level is not elevated at the time adrenalin inhibits insulin gastric hypermotility.

Spontaneous motility of the empty stomach displays a striking similarity to insulin hypermotility. This is true to such a degree that physiologically produced insulin might be considered a factor controlling spontaneous gastric motility. Since post-insulin motility is completely inhibited by intravenously injected glucose but spontaneous motility is not modified by such injections, insulin production cannot be the controlling factor in spontaneous gastric motility. Furthermore, this difference in behavior

towards glucose affords a ready method of distinguishing post-insulin hypermotility from spontaneous motility of the empty stomach. Why intravenous glucose completely inhibits gastric motility in the insulinized animal but is without effect on spontaneous gastric motility has not been satisfactorily explained. Psychic factors may play a rôle for animals usually become restless when the marked insulin hypoglycemia is relieved by intravenous glucose.

Quigley, Hallaran and Barnes (1932) have shown that blood sugar levels or carbohydrate metabolism is not strikingly different in normal and chronic vagotomized dogs. This may be taken as further evidence that the depressed gastric tone and motility observed in vagotomized animals cannot be related to abnormalities in carbohydrate metabolism.

SUMMARY

Investigating the theory that spontaneous motility of the empty stomach is inversely related to the blood sugar level, we have in general obtained experimental results not in agreement with this theory.

We failed to modify gastric motility in normal or vagotomized dogs by the intravenous injection of glucose. Such injections also were without effect on motility of the terminal ileum or colon. The spontaneous hypoglycemia occurring several hours after glucose administration was without constant effect on motility of the stomach, ileum or colon. Glucose, cane sugar or lactose introduced into the empty stomach produced gastric inhibition in the normal or vagotomized dog. This inhibition was apparently not related to the production of hyperglycemia but appeared to be a reflex from the duodenum. Carbohydrates introduced into the duodenum lead to a similar gastric inhibition.

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FURTHER STUDIES OF THE FOLLICULAR-CORPUS LUTEUM HORMONE RELATIONSHIP IN THE RABBIT[1]

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In a previous paper (Hisaw and Leonard, 1), it was pointed out that the production of a progestational endometrium in the uterus of a castrate rabbit required the combined action of the follicular and corpus luteum hormones in which the follicular hormone had to exert its influence first. This "one-two" relationship was previously demonstrated for the decidual reaction in the uterus of rats and guinea pigs by Weichert (2) and for the corpus luteum hormone "relaxin" by Hisaw (3), (4) and Fevold et al. (5). This synergistic mode of action of the two ovarian hormones to produce the uterine changes in the rabbits has been upheld by Allen (6), Clauberg (7), (8) and Parkes (9). It was also shown in the previous communication (1) that the corpus luteum hormone "corporin" (Hisaw) "progestin" (Corner) responsible for progestational changes could not maintain indefinitely the characteristic histological picture of early pregnancy in the uterus of castrate rabbits. Also it was demonstrated by injections of follicular hormone and corporin that it was possible to prevent the progestational modification of the uterus by sufficient quantities of the follicular hormone. In this paper we wish to present further data on the oestrin-corporin relationship and to discuss certain other physiological aspects which have developed from the experiments to be reported.

MATERIAL AND METHODS. The corpus luteum hormone responsible for the progestational reaction has been termed "corporin" in this laboratory and is the same active substance used to produce premenstrual changes of the endometrium in the uterus of castrate monkeys (Hisaw, Fevold and Meyer, 10). It is also essentially similar to Allen's (11) preparation "progestin."

Since the discovery of the separation of the corpus luteum hormones, (Fevold et al., 12) a choice of methods of preparation of corporin may be made depending on whether or not it is desired first to recover the relaxative hormone. These methods are given in full in a recent publication

[1] Aided in part by grants from the National Research Council, Committee on Problems of Sex, The University of Wisconsin Research Fund, and the Wisconsin Alumni Research Foundation.

(Fevold et al., 13). The corporin which was used in the following experiments was extracted from the corpus luteum tissue by hot acid alcohol and purified as described (13). The amount of corporin necessary to produce a good progestational modification in the uterus of rabbits castrated in heat is equivalent to 25 to 50 grams of fresh tissue. This preparation was practically free of oestrin in that 25 to 50 grams equivalent were not sufficient to bring a castrated female rat into heat although 75 grams apparently contained sufficient amounts to do so. It is preserved in 100 per cent alcohol in stock solution, dissolved in corn oil as a vehicle when ready for injections, and only sufficient quantities are prepared in oil to last for five days. Several batches of corporin were employed in these experiments but in attempting to examine the relationships of oestrin and corporin in a partially quantitative way a single batch was used. The follicular hormone employed was oil soluble theelin and was carefully restandardized just previous to using.[2]

Sexually matured female rabbits ranging in weight from $2\frac{1}{2}$ to $3\frac{1}{2}$ kilograms were isolated for 15 days or more before they were used in these experiments. Thirty-five animals were employed in the tests. Injections of corporin were made twice daily, follicular hormone once daily, and the two hormones when used together were always injected separately. In describing the degree of uterine glandular development, it was decided to abide as closely as possible to the standard set down by Allen (11) which consists of four degrees, +1 to +4. A rabbit unit of the active principle is defined as that amount of extract which will produce in 5 days a uterine modification, in an adult rabbit castrated in heat, equal to that normally occurring on the 6th to 8th day of pregnancy (+3 to +4).

Experimental. It was pointed out in a previous paper (1) that corporin alone when given over an extended period, would not maintain the characteristic histological picture of early pregnancy as produced in 5 days. This experiment has been extended further to confirm our original suggestion. For example, corporin was injected in doses of 1 to 4 rabbit units for each 5 days for a total of 15 days, samples of the uterus being removed and sectioned every 5 days. The results clearly showed that no matter how active the extract, it was impossible to prolong the modification beyond 10 days. A collapse of the glands could be seen by the 10th day and retrogressive changes in the cells lining the lumen of the glands of the uterus could be demonstrated. By the 15th day, the uterine glands had almost completely disappeared, their lumens had become occluded, and numerous leukocytes had invaded the endometrium. The tall columnar epithelium so characteristic of the active glands became very low and the ends of the cells much frayed out. The nuclei of the epithelial layer

[2] The theelin was kindly furnished by Parke, Davis & Co.

instead of being elongated as in the first five days of treatment became rounded and pycnotic by the 15th.

TABLE 1

*Effect of varying doses of theelin and corporin on progestational modifications of the uterine endometrium of rabbits**

RABBIT	TREATMENT (5 DAY PERIODS)	RESULT OR DEGREE OF UTERINE MODIFICATION
RA130	1. 50 grams tissue equivalent	1. +3
RA134**	1. 75 grams tissue equivalent 2. Injections continued 5 more days at same level	1. +4 2. Glands regressing
RA131	1. 1 Rb.U. corporin plus 50 R.U. theelin	1. In heat
RA132	1. 1 Rb.U. corporin plus 25 R.U. theelin	1. In heat
RA143	1. 1 Rb.U. corporin plus 25 R.U. theelin 2. Injections continued 5 more days at same level	1. +1 2. Few glands, endometrium in heat
RA147	1. 1 Rb.U. corporin plus 25 R.U. theelin 2. Injections continued 4 days, theelin stopped, corporin injected 5 days	1. In heat 2. +2 to +3
RA144	1. 1 Rb.U. corporin plus 10 R.U. theelin 2. Injections continued 5 more days same level	1. In heat 2. In heat
RA148	1. 1 Rb.U. corporin plus 10 R.U. theelin	1. In heat
RA150	1. 1⅓ Rb.U. corporin plus 10 R.U. theelin 2. 2⅔ Rb.U. corporin plus 10 R.U. theelin	1. +2 to +3 2. Glands regressing
RA146	1. 3⅓ Rb.U. corporin plus 25 R.U. theelin	1. +2
RA149	1. 2 Rb.U. corporin plus 25 R.U. theelin 2. Corporin same 5 more days; theelin 5 R.U.	1. +2 to +3 2. Glands regressing
RA153	1. 5⅓ Rb.U. corporin plus 25 R.U. theelin	1. +2 to +3

* Rabbits castrated in heat, injections begun immediately.

** 75 grams = 1 Rb.U. All experiments in table 1 from same extract.

Knaus (14) while studying the changes in the sterile horn of unilaterally pregnant rabbits found that the endometrium also went through similar degenerative stages but by the time of parturition, had returned to the

oestrous condition. Courrier and Kehl (15) and Courrier (16) have shown that fresh corpora lutea produced with anterior pituitary material in the unilaterally pregnant rabbit could not induce new glandular formation in the sterile horn.

Empirically, the fact that oestrin could over-ride the corpus luteum hormone when both were injected simultaneously into castrate rabbits was demonstrated previously by Hisaw and Leonard (1). In the experiments reported here an attempt is made to establish a quantitative balance between the two and to demonstrate their action when competing for physiological control of the endometrium. The results given in table 1 are from the same stock solution so that this series of experiments was performed without changing extracts. It was found that 50 grams equivalent of this extract would produce a +3 reaction in the uterus of a rabbit castrated in heat (RA130), but in order to guard against any

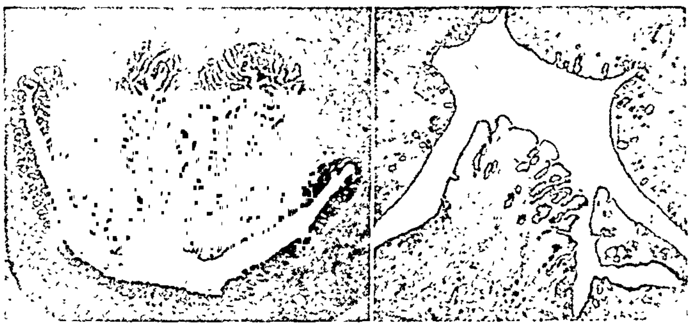

Fig. 1 Fig. 2

Fig. 1. RA150–1. Rabbit castrated in heat, received 1⅓ rabbit units of corporin plus 10 R. U. of theelin. Corpus luteum effects indicated.

Fig. 2. RA144–1. Rabbit castrated in heat, received 1 rabbit unit of corporin (75 grams tissue equivalent) plus 10 R. U. of theelin. Oestrin effects indicated.

possible variations, an equivalent of 75 grams of tissue giving a +4 reaction (RA134) was taken as the rabbit unit. Several preliminary trials were made before the final series of experiments in order to approximate the amount of theelin necessary to prevent the action of a given amount of corporin.

One rabbit unit of corporin (75 grams equivalent) was injected with 50, 25 and 10 R.U. of theelin for 5 days into rabbits castrated in heat. Ten rat units of theelin seemed to be the smallest amount to prevent the action of one rabbit unit of corporin, for in one of the animals receiving this amount (RA148) the cells of the uterine glands had only just begun to undergo the characteristic progestational change (fig. 1). But when a third of a rabbit unit more of corporin was injected with the 10 R.U. of theelin, a definite and unmistakable progestational reaction resulted

(RA150) (fig. 2). The injections were continued for 5 more days and the dosage of corporin was doubled, yet in spite of this, the usual degenerate changes in the uterine glands occurred.

When the dosage of corporin was raised to $3\frac{1}{3}$ rabbit units and the theelin to 25 R.U. the effect of corporin unmistakably superseded that of the theelin to give a +2 reaction (RA146). Similarly, two rabbit units of corporin and 25 R.U. of theelin gave a positive progestational modification (RA149). By lowering the dosage of theelin to 5 R.U. and keeping the amount of corporin constant for 5 more days, it was found impossible to prevent the onset of the degenerative changes on the 10th day. In the last case (RA153) a massive dose of $5\frac{1}{3}$ rabbit units of corporin was given along with 25 R.U. of theelin which resulted in a +2 to +3 modification. It was observed that whenever the combined injections were made and the corporin was more powerful in its influence, the end result was never better than a possible +3 reaction. Upon histological examination of the uterus of animals injected with both preparations simultaneously, a greater engorgement of the lymphatics and capillaries could be observed. This was indicated particularly when the theelin was more effective than corporin in the test animal.

An interesting result was obtained in rabbit RA147 which received one rabbit unit of corporin plus 25 R.U. of theelin for 5 days and then had a piece of the uterus removed and sectioned: The histological examination revealed an endometrium of an animal in heat with very much enlarged capillaries and lymphatic channels. The dosage of oestrin and corporin was kept constant for 4 more days, then the oestrin injections were stopped and the corporin continued for 5 more days at the same level. The result was a progestational endometrium of +2 to +3. Evidently, the oestrous hormone kept the uterus tuned up, so to speak, in the presence of sufficient corporin which alone could have produced a typical reaction in the first five days. Such experiments will explain much of the so-called antagonism of the ovarian hormones.

Further, it has been demonstrated by a number of workers that oestrin will terminate the early stages of pregnancy quite easily. Courrier (15) has shown that while small amounts of oestrin will terminate the early stages of pregnancy in rabbits, it takes large amounts (500 to 700 R.U.) to prevent the normal progestational changes which appear in the endometrium in the first 5 to 8 days. To test this, four rabbits were bred and given 50, 100, 200, and 500 R.U. of theelin respectively over a period of five days and pieces of the uteri were removed and sectioned. The results confirmed those of Courrier. No effects on the endometrium of the rabbits receiving 50 and 100 R.U. respectively could be determined, the one receiving 200 R.U. gave a +1 to +2 reaction. However, the uterus of the rabbit injected with 500 R.U. of theelin was completely negative,

the corpora lutea being unable to produce the modification in the presence of such large amounts of the oestrus-producing hormone. It is also difficult to inhibit progestational development with theelin during pseudopregnancy as shown by the following experiment. A rabbit which was in heat received 300 R.U. of theelin in three days, was allowed to copulate and then was given 125 R.U. during the next 5 days. A lutein reaction in the endometrium of +2 to +3 resulted in spite of the preliminary treatments of large amounts of theelin. These results show (1) that large doses of theelin may not inhibit ovulation, and (2) that there is a significant difference between the amounts of theelin needed to blot out the normal progestational picture as compared to the amount it takes to over-ride a rabbit unit of injected corporin.

Since it was possible to inhibit the normal progestational modification with large amounts of theelin, the question arose as to whether or not the

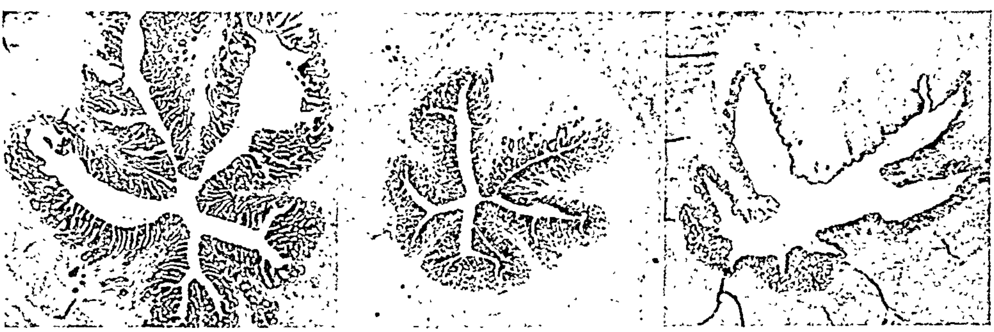

Fig. 3 Fig. 4 Fig. 5

Fig. 3. RA88–1. Rabbit castrated in heat, injected with corporin for 5 days. Progestational changes present.

Fig. 4. RA88–2. Same animal on tenth day, the injections of corporin continued at same level. Note involution of glands.

Fig. 5. RA88–3. Same animal on fifteenth day; the injections of corporin continued at same level. Marked involution of glands.

concentration of oestrin was sufficiently high in the normal rabbit in heat to readily over-ride any injected corporin. If the rabbit has been isolated for 15 to 20 days, it is possible to elicit an apparently normal development of the uterine glands in 5 days with corporin. For example, RA138 was given 2 rabbit units of corporin for 10 days and a section of the uterus removed on the 5th and 10th day. The endometrium was diagnosed as +4 on the 5th day and degenerative changes, though not marked, had begun by the 10th day. But this reaction cannot be considered as positive if by handling the animals or in some manner ovulation occurs because, in such cases, the animals' own corpora lutea would defeat the purpose of the test. However, the ovaries of all animals so injected contained small

follicles and never any recently formed corpora lutea. It was also interesting to note that ovulation could be induced in rabbits on the 5th day of corpus luteum treatment by the injection of the urine of pregnant women after the manner described by Friedman (17).

Klein (18) also has been able to induce progestational modifications in the endometrium of non-castrate female rabbits but claims that it is more difficult to do so. To test this, a rabbit unit of corporin which would produce a +3 reaction in the uterus of a rabbit castrated in heat was injected into a non-castrated animal with the result of only a +1 reaction. However, when the dose was raised to $1\frac{1}{2}$ rabbit units a +4 reaction resulted. This experiment is very suggestive of the nature of the follicular hormone content of the rabbit in heat. A further discussion of these several problems is given below.

Discussion. From the first series of experiments in which rabbits castrated in heat received corporin for 15 days, it is seen that regression of the endometrial glands occurs in spite of ample amounts of the hormone (figs. 3, 4, 5). This is not at all surprising if one considers the follicular hormone as a growth promoter and sensitizer of the uterus and corporin as a modifier of structures already formed by the follicular hormone. The effects of the follicular hormone alone in producing the hyperemic condition of the uterus and growth of both the muscle and the endometrium are well known. When the source of this hormone is removed from the animal as after castration, retrogressive changes toward the castrate condition follow, but if the lutein hormone is present, it can modify the uterus during the process. This is probably what occurs normally during the early pregnant or pseudopregnant period of the rabbit's cycle in which the follicular hormone is probably at a very low ebb shortly following ovulation, and the lutein hormone exerts its influence without further interference from the oestrus-producing hormone. It is suggested that progestational "modification" be substituted for "proliferation" used heretofore as the latter does not signify the nature of action of the luteal hormone.

Knaus (14) claims that the uterine glands of the pregnant rabbit secrete longer than the pseudopregnant animal as the result of the larger size of the corpora lutea of pregnancy and that the retrogressive processes of the glands in the pregnant animal commence about the 15th to the 17th day of gestation. In the castrate animal injected with corporin, the atrophic changes are first seen on about the 10th day of injections but by the 15th day the glands have for the most part disappeared. It is the opinion that the difference in appearance of the degenerative changes in the experimental and pregnant animals is more apparent than real because of the conditions of the experiment. It must be remembered that in the experimental animal, from the time of castration in heat, corporin is available

in very high concentration while in the pregnant animal some time elapses before the corpora lutea reach a sufficient size to be fully active. This may account for the apparent earlier involutionary process of the glands in the injected animal. In the pseudopregnant rabbit, other factors as well may enter in to cause the earlier degenerative changes to appear, i.e., the onset of the next heat period.

Courrier (15) has demonstrated that the production of fresh corpora lutea in the ovaries of a unilaterally pregnant rabbit would not induce anew the endometrial changes characteristic of the first stages of pregnancy. This seems to be of great significance since Allen and Corner (19) have demonstrated that progestin will maintain pregnancy to term in a rabbit castrated 18 hours after copulation. The same extract that will produce the early changes of pregnancy is necessary for the maintenance of pregnancy and the life of the placenta. These facts make one speculate as to other possible factors in the lutein hormone necessary for the maintenance of pregnancy or the relation of the placenta to this hormone.

Further evidence that oestrin in the doses given would not prolong the progestational picture in the endometrium is observed from the experiments on simultaneous injections of corporin and theelin. The degenerative changes can be seen on the 10th day in spite of the presence of both hormones. More interesting still, is the fact that the result to be expected from simultaneous injections of both hormones depends on the amount of either given. In other words, a physiological antagonism exists between the two. The word "antagonism' has been used rather loosely in speaking of hormone interrelations but now it is possible, at least in this case, to further explain the nature of this opposing action.

If the amount of follicular hormone is physiologically greater than corporin, the endometrium remains in the oestrous state; if the corporin is physiologically greater in amount than the follicular hormone, the glands undergo their modification as they would normally. The two hormones then react towards one another as two different substances competing for the cells of the endometrium, either to maintain them as they are (pre-secretory phase) or modify them into the secreting phase characteristic of early pregnancy. A good example to illustrate the nature of the interaction of corporin and theelin is to be found in rabbit RA147 which received simultaneous injections of both hormones. The dosage was so given that at the end of the first five days, the theelin was stronger than the corporin and the oestrous condition was maintained in the uterus. This treatment was continued for 4 more days making a total of 9 and then the theelin was discontinued and the corporin administered at the same level for 5 more days. The result was that with the discontinuance of theelin, the corporin could still produce its modifications on the endometrium.

The literature contains many conflicting statements regarding antagonism of the ovarian hormones. Courrier (20) has shown the antagonistic reaction of oestrin on the corpora lutea of the guinea pig using the production of deciduomata as a test point. Angelvitz and Sterling (21) have stated there is no antagonism between progestin and oestrin using the vaginal mucification of the rat as a test. Patel (22) studying the same reaction has a very suggestive explanation of the relation of a lutein hormone and oestrin in that the lutein hormone reduces the activity of the vagina to oestrin and that it is the oestrous hormone which produces mucification. Parkes (9) claims that oestrin is not antagonistic to the uterine changes and that it aids in the progestational reaction. This is perhaps true in part but nevertheless the effects of corporin can be clearly destroyed provided sufficient oestrin is present.

The work of Courrier (16) and others in injecting large doses of oestrin to over-ride the action of normal corpora lutea has been confirmed. It is interesting to compare the amounts of oestrin that prevent the progestational development in the normal animal (500 R.U.) and the amount needed to override a rabbit unit of corporin (10 R.U.). The reason for this cannot be explained at present but one suggestion is offered in that the animal's own corpora lutea may produce many times the amount of corporin needed to affect a progestational reaction.

It has been demonstrated also that progestational changes can be produced with corporin in an adult non-castrate rabbit in heat. Klein (18) claims that it is more difficult to produce the reaction in such animals but does not say how much more hormone is needed. In the experiments reported, it was apparently necessary to use only a half of a rabbit unit more of corporin to produce a positive reaction. Klein also states that prolonged injections caused follicular atresia but in these experiments, the amount of corporin needed to produce a uterine modification in 5 days did not interfere with the ability to induce ovulation following the proper stimulation.

The experiment of inducing progestational modifications in the adult non-castrate rabbit permits one to speculate as to the concentration of the follicular hormone in the rabbit during heat. Assuming that it takes only a third of a rabbit unit more of corporin to override 10 R.U. of theelin in a castrate animal and only a half of a rabbit unit more to produce the reaction in the non-castrate female, one could conjecture that the concentration of the follicular hormone was a little better than 10 R.U. in the rabbit in heat. This is probably by no means accurate but at the same time does not seem entirely fantastic.

Our own studies and those of others indicate that the endometrium of the rabbit uterus cannot respond to both corporin and theelin at the same time but shows the effects of one or the other depending upon which

hormone has a quantitative advantage. The point at which the shift occurs is not sharp and in several respects resembles the results of a mass reaction. It also seems that the endometrium is conditioned by theelin and is depleted by secretion in response to corporin and that both processes cannot occur simultaneously in the same gland cell. We also have unpublished data which suggest that this explanation may also apply to the premenstrual endometrium of monkeys.

SUMMARY

1. It has been definitely shown that a quantitative relationship exists between oestrin and corporin in the production of endometrial changes characteristic of early pregnancy.

2. If oestrin and corporin are injected simultaneously into castrate rabbits, the result to be expected depends on the higher dosage of either hormone and the apparent antagonism is explained on the basis of the two hormones competing physiologically for the endometrium.

3. The oestrous hormone can hold the action of injected corporin in abeyance for an extended period (9 days at least) after which, if the dosage of oestrin is lowered and corporin treatment continued, the effect of corporin becomes manifested.

4. It takes many more times the amount of oestrin to obliterate the characteristic modification in the endometrium of early pregnancy than it does to overpower the effects of a rabbit unit of corporin capable of producing a histological picture equally as good in a castrate animal.

5. It takes slightly more corporin to produce a progestational modification in a non-castrate female rabbit in heat than it does to produce a comparable change in the uterus of a rabbit castrated in heat and then treated.

6. Corporin alone or with the addition of oestrin does not seem able to maintain the progestational modification indefinitely.

7. The amount of corporin which in 5 days will produce a good progestational modification in a non-castrate rabbit in heat will not inhibit ovulation following the proper stimulation.

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THE TRANSFER OF BICARBONATE BETWEEN THE BLOOD AND TISSUES CAUSED BY ALTERATIONS OF THE CARBON DIOXIDE CONCENTRATION IN THE LUNGS

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Henderson and Haggard (1918a, b) showed by acute experiments on dogs that alterations in the carbon dioxide tension of the alveolar air induced by breathing air enriched by carbon dioxide, or by over-ventilation, were followed by alterations in the bicarbonate reserve of the blood. The duration of these experiments was measured by minutes and must not be confused with experiments in which the duration is measured by days. The changes referred to were made apparent by a change in the level of the carbon dioxide absorption curve of whole blood: when the carbon dioxide tension of the alveolar air was raised, the level of the carbon dioxide absorption curve was raised; and when the alveolar carbon dioxide tension was lowered, the level of the carbon dioxide absorption curve was lowered. In this manner the ratio $\left(\frac{H_2CO_3}{BHCO_3}\right)$ tended always to approximate the normal value, bicarbonate passing from the tissues into the blood when the dissolved carbon dioxide was abnormally high, and from the blood into the tissues when the dissolved carbon dioxide was abnormally low. This principle has been widely accepted as a compensatory device, the function of which is to maintain the hydrogen ion concentration of the blood at the normal level.

Collip and Backus (1920) examined the plasma from 15 human subjects taken after 20 to 40 minutes of forced breathing, accompanied by an average fall in the alveolar carbon dioxide tension from the normal of 46 mm. to 22.5 mm. They found that plasma taken before over-ventilation combined with 66.6 volumes per cent of carbon dioxide when equilibrated with alveolar air, and immediately following the period of over-ventilation combined with only 55.6 volumes per cent of carbon dioxide when equilibrated at the same carbon dioxide tension. The results of these experiments on man are in substantial agreement with those of Henderson and Haggard (1918b) on dogs.

Grant and Goldman (1920) found that when the alveolar carbon dioxide tension in man was lowered from 41 to 23 mm. of mercury by voluntary

forced breathing, the carbon dioxide combining capacity of the plasma fell from 59.5 to 44.9 volumes per cent.

Davies, Haldane and Kennaway (1920) found that when the alveolar carbon dioxide tension in man was increased by breathing 5.2 to 6.4 per cent carbon dioxide in air for a period of 2 hours, the level of the carbon dioxide absorption curve was unaltered; nor was any change produced by lowering the alveolar carbon dioxide tension to about 11 mm. by voluntary forced breathing for 1 hour.

In view of the assumed efficacy of this mechanism in maintaining the acid base balance of the body, we have undertaken a series of experiments on both dogs and cats, which we hoped would throw further light on the extent and causes of the changes observed. In all of our experiments we found that the changes were either non-existent or in the reverse direction of those reported by Henderson and Haggard.

We shall avoid using the term "alkali reserve" in this paper, since the alkali in the blood is always in use and never in reserve. For our purposes the term "bicarbonate level" is more explicit, because any change in the alkali content of the blood will be made apparent by a shift in the level of the carbon dioxide absorption curve.

Method. The animals used were cats and dogs. A 10 per cent solution of barbital sodium was allowed to run slowly from a burette into the femoral vein, which had been exposed under ether anesthesia. The dose varied from 0.3 to 0.4 gram per kilo of body weight. A cannula was inserted in the trachea to facilitate the withdrawal of alveolar air samples, and another in the femoral or carotid artery for the withdrawal of blood samples. The animal was then permitted to rest for at least 3 hours before the first sample of blood was taken. This interval of time was considered necessary in order that a condition of stable equilibrium might be attained following the administration of the anesthetic and the operative procedure.

The alveolar carbon dioxide tension was raised by the inhalation of a gas mixture containing about 10 to 11 per cent carbon dioxide, 25 per cent oxygen, and nitrogen. The gas mixture was allowed to flow into a spirometer, from which the gas was withdrawn by the animal through respiratory valves.

The alveolar carbon dioxide tension was lowered by over-ventilation, by using either a positive pressure pump which was connected to the tracheal cannula or by using a respirator which induces the normal movements of the chest, similar to that described by Shaw and Drinker (1929) for the administration of artificial respiration to children. The rate of the apparatus was adjusted to fill the lungs about 25 times per minute.

Alveolar air was withdrawn for carbon dioxide determination by the method described by Shaw and Messer (1930). Samples were taken while

the animal was breathing normally and again during the period of altered alveolar carbon dioxide tension.

The blood samples were oxalated in air and then immediately introduced into tonometers containing about 25 per cent oxygen and carbon dioxide in such concentration that, after the equilibration had been completed, a sample of gas from the tonometer had a carbon dioxide tension of 40 ± 3 mm. Hg. The carbon dioxide content of the blood, as determined by the method of Van Slyke and Neill (1924), was then corrected to that which would exist if the carbon dioxide tension were exactly 40 mm. The correction thus applied, which was based upon the average slope of the carbon

TABLE 1

Control determinations of the carbon dioxide content of blood following equilibration at a given tension of carbon dioxide

EXPERIMENT 28			EXPERIMENT 30			EXPERIMENT 49		
Weight 9.3 kgm.			Weight 11.2 kgm.			Weight 7.4 kgm.		
Minutes	CO_2 at 40 mm.	Averages	Minutes	CO_2 at 40 mm.	Averages	Minutes	CO_2 at 40 mm.	Averages
	Air			Air			Air	
	vols. per cent			*vols. per cent*			*vols. per cent*	
			5	43.2				
30	49.2		35	43.1		30	41.7	
60	49.3		65	43.2		60	42.9	
82	48.5		95	42.5		90	43.2	
111	49.4		125	42.6		120	42.7	
140	49.0	49.1	155	42.8	42.9	150	42.3	42.5

TABLE 2

Control determinations of the constancy of the carbon dioxide combining capacity of the blood of dogs which are resting under experimental conditions

EXPERIMENT 22			EXPERIMENT 27			EXPERIMENT 29		
Weight 8.4 kgm.			Weight 15.2 kgm.			Weight 12.7 kgm.		
Minutes	CO_2 at 40 mm.	Averages	Minutes	CO_2 at 40 mm.	Averages	Minutes	CO_2 at 40 mm.	Averages
	Air			Air			Air	
	vols. per cent			*vols. per cent*			*vols. per cent*	
0	43.4		0	39.4		0	43.2	
30	39.6		20	41.2		30	42.5	
60	43.8		40	42.1		60	43.8	
90	43.5		60	42.0		90	43.0	
120	43.4		80	42.1		120	43.3	
150	42.8	42.8	100	41.9	41.4	150	43.7	43.2

dioxide absorption curve of the animal's blood, was subject to an error so slight as to be negligible.

Since the determination of the carbon dioxide content of blood following equilibration at a given tension of carbon dioxide involves several procedures, each of which is subject to error, we have shown the results of three control experiments in table 1 so that the effective error may be correctly appraised. About 60 cc. of blood were withdrawn from a dog and 10 cc. introduced into each of 5 or 6 tonometers. To test for the possible formation of lactic acid due to standing at room temperature, the samples were equilibrated and transferred to the mercury collecting tubes at intervals of 30 minutes. The last sample was, therefore, exposed to the effect of room

temperature for about 145 minutes longer than the first sample. No change in its carbon dioxide combining capacity was observed. The process of equilibration plus carbon dioxide analysis is subject to an error of ±0.7 volume per cent, or a disagreement of 1.5 volumes per cent between the minimum and maximum values of a series. The effect of an error, however, is greatly minimized by the fact that the results of an experiment are given in terms of averages taken from two or more observations.

The constancy of the bicarbonate level in the blood under the conditions of our experiment is shown in table 2. In these control experiments dogs were anesthetised, prepared, and allowed to rest for 3 hours before the first

TABLE 3

The respiratory volume of dogs under experimental conditions

EXPERIMENT	WEIGHT	NATURAL ALVEOLAR CO_2	NATURAL RESPIRATORY RATE PER MINUTE	RESPIRATORY VOLUME (CC. PER MINUTE PER KILO BODY WEIGHT)		
				Natural	High CO_2	Over-ventilation
(1)	(2)	(3)	(4)	(5)	(6)	(7)
	kgm.	*mm.* Hg				
44	12.2	46	8	158		485
43	12.5	49	8	172	590	
37	18.9	45	14	200	980	
47	9.0	46	25	234		1,190
45	11.4	46	16	246		780
56	9.9	46	19	256		1,160
31	19.6	43	19	260	653	
35	18.0	40	25	272	1,280	
36	15.4	40	22	280	1,240	
34	13.5		22	288	845	
32	10.2	43	20	304	1,285	
46	12.2	35	29	360		1,230
48	8.5	34	31	360		1,300
33	17.0	38	60	512	1,310	

blood sample was taken, in the manner already described. Blood samples were then taken while the dog was breathing normally at intervals during a period of time equivalent to the duration of our regular experiments to determine the range of variation in the bicarbonate level which might be expected to occur quite irrespective of alterations in the alveolar carbon dioxide tension. While the prevailing level remains very steady, the extreme variation is greater than the analytical error, owing to an occasional fall in the bicarbonate level which is only transitory in effect.

It was found that the average rectal temperature taken directly after the administration of the anesthesia was 39.0°C. in the dog and 38.3°C. in the cat. These temperatures were held constant within ±0.5°C. through-

out the experiment by the use of artificial heat. After a few minutes of hyperventilation, caused by the inhalation of carbon dioxide or by enforced over-ventilation, the body temperature started to fall, but was instantly checked by increasing the external heat.

The temperature of the bath in which the blood was equilibrated was held at 37°C. Since this is 1.3 to 2.0°C. lower than the body temperature of the animal from which the blood was taken, the carbon dioxide combining capacity of the blood as determined by us must be subjected to a slight correction. But, owing to the fact that all the data presented are concerned with the relative values of the carbon dioxide combining capacity and not with the absolute values, the correction becomes superfluous.

In table 3 the respiratory volume of the dogs is given in terms of cubic centimeters per minute per kilo of body weight. The data are arranged in order of the increasing values of the natural respiratory volume (column 5), which was determined just prior to the induced change in the alveolar carbon dioxide tension (columns 6 and 7). It will be observed that the alveolar carbon dioxide tension varies from 49 to 34 mm. of mercury. We do not know the alveolar carbon dioxide tensions of the normal intact dog, but it is probable that the fluctuations which appear under the conditions of our experiments are greater than the fluctuations which would occur in the intact dog. Immediately following the intravenous injection of barbital sodium the respiratory rhythm is usually temporarily disturbed. It may, however, even after 2 or 3 hours, remain abnormally rapid or abnormally slow, which in turn is responsible for the extremes which occur in the alveolar carbon dioxide tension.

The response to over-ventilation (column 7) was governed entirely by the respirator. The response to the carbon dioxide inhalation (column 6), on the other hand, depends upon the respiratory center. Low responses to the inhalation of carbon dioxide were observed in experiments 31, 34 and 43. It was noted that dog 31 was more profoundly anesthetised than the other animals, and that number 34 was recovering from distemper. These may have been contributing causes of the inadequate response to the carbon dioxide stimulus.

Experimental results. *The effect of raising the alveolar carbon dioxide tension upon the bicarbonate level of blood.* The effect upon dogs' blood is given in table 4. The manner of tabulating our data can best be shown by reviewing in detail experiment 32 in table 4. The time in minutes following the first blood sample is given in column 1. Two blood samples were taken with 30 minutes intervening while the dog was breathing room air. Thirty-one minutes after the first sample the respiratory valves were connected to a spirometer containing the carbon dioxide gas mixture. The alveolar carbon dioxide tension was raised from 43 mm. to 83 mm., and the average bicarbonate level of the blood fell from 46.2 volumes per cent

TABLE 4

The transfer of bicarbonate between the blood and tissues of dogs, caused by increasing the carbon dioxide concentration in the lungs

EXPERIMENT 32			
Weight 10.2 kgm.			
Minutes	CO_2 at 40 mm.	Averages	Alveolar CO_2
	Air		
	vols. per cent		*mm. Hg*
0	45.7		
30	46.8	46.2	43.0
31	High CO_2		
35	43.4		
45	43.2		
58	44.6		
73	43.2	43.6	83.0
74	Air		
78	46.1		
93	45.5		
108	45.9	45.8	

EXPERIMENT 33			
Weight 17 kgm.			
Minutes	CO_2 at 40 mm.	Averages	Alveolar CO_2
	Air		
	vols. per cent		*mm. Hg*
0	43.5		
30	44.9	44.2	38.0
35	High CO_2		
40	42.5		
50	42.3		
65	42.6		
74	42.8	42.5	92.0
75	Air		
80	46.1		
95	46.1	46.1	

EXPERIMENT 34			
Weight 13.5 kgm.			
Minutes	CO_2 at 40 mm.	Averages	Alveolar CO_2
	Air		
	vols. per cent		*mm. Hg*
0	44.6		
30	43.7	44.1	
35	High CO_2		
40	41.4		
50	40.4		
65	41.0		
74	41.7	41.1	
75	Air		
80	43.4		
95	45.0		
110	44.3	44.2	

EXPERIMENT 35			
Weight 18 kgm.			
Minutes	CO_2 at 40 mm.	Averages	Alveolar CO_2
	Air		
	vols. per cent		*mm. Hg*
0	46.3		
30	45.4	45.8	40.0
35	High CO_2		
40	44.0		
50	43.7		
65	44.2		
74	44.0	43.9	85.0
75	Air		
80	46.5		
95	46.9	46.7	

EXPERIMENT 36			
Weight 15.4 kgm.			
Minutes	CO_2 at 40 mm.	Averages	Alveolar CO_2
	Air		
	vols. per cent		*mm. Hg*
0	43.5		
30	43.2	43.3	40.0
35	High CO_2		
40	40.1		
50	40.9		
65	40.7		
74	39.6	40.3	85.0
75	Air		
80	41.2		
95	40.8		
110	41.7	41.2	

EXPERIMENT 37			
Weight 18.9 kgm.			
Minutes	CO_2 at 40 mm.	Averages	Alveolar CO_2
	Air		
	vols. per cent		*mm. Hg*
0	41.6		
30	42.9	42.2	45.0
35	High CO_2		
40	41.6		
50	40.9		
65	39.2		
74	40.9	40.6	90.0
75	Air		
80	44.7		
95	44.9		
110	44.9	44.8	

EXPERIMENT 43			
Weight 12.5 kgm.			
Minutes	CO_2 at 40 mm.	Averages	Alveolar CO_2
	Air		
	vols. per cent		*mm. Hg*
0	Lost		
30	46.9	46.9	49.0
35	High CO_2		
40	43.7		
66	43.2		
76	43.9		
		43.6	89.0
77	Air		
84	46.7		
99	47.2		
114	46.2	46.7	

EXPERIMENT 31			
Weight 19.6 kgm.			
Minutes	CO_2 at 40 mm.	Averages	Alveolar CO_2
	Air		
	vols. per cent		*mm. Hg*
0	40.4		
30	40.2	40.3	43.0
31	High CO_2		
35	39.8		
45	40.0		
57	40.2		
70	39.9	40.0	83.0
72	Air		
75	40.6		
90	41.1		
105	41.5	41.0	

TABLE 5

The transfer of bicarbonate between the blood and tissues of cats, caused by increasing the carbon dioxide concentration in the lungs

EXPERIMENT 11				EXPERIMENT 12				EXPERIMENT 13			
Weight 4.5 kgm.				Weight 4 kgm.				Weight 3.6 kgm.			
Minutes	CO_2 at 40 mm.	Averages	Alveolar CO_2	Minutes	CO_2 at 40 mm.	Averages	Alveolar CO_2	Minutes	CO_2 at 40 mm.	Averages	Alveolar CO_2
	Air				Air				Air		
	vols. per cent		*mm.* Hg		*vols. per cent*		*mm.* Hg		*vols. per cent*		*mm.* Hg
0				0	45.0			0	45.4		
30	45.6	45.6	30.0	21	45.3	45.1	32.0	15	43.5	44.4	33.0
32	High CO_2			28	High CO_2			25	High CO_2		
45	39.2			43	39.8			30	41.8		
60	38.4			58	40.3			55	41.3		
75	39.4			73	40.5			70	40.7		
90	38.2	38.8	90.0	88	40.7	40.3	91.0			41.2	90.0
92	Air			90	Air			80	Air		
120	46.0			133	43.8			85	45.2		
140	46.4	46.2		148	43.7	43.7		115	44.2	44.7	

EXPERIMENT 38				EXPERIMENT 40				EXPERIMENT 41				EXPERIMENT 42			
Weight 5.6 kgm.				Weight 5.5 kgm.				Weight 4.7 kgm.				Weight 3.8 kgm.			
Minutes	CO_2 at 40 mm.	Averages	Alveolar CO_2	Minutes	CO_2 at 40 mm.	Averages	Alveolar CO_2	Minutes	CO_2 at 40 mm.	Averages	Alveolar CO_2	Minutes	CO_2 at 40 mm.	Averages	Alveolar CO_2
	Air				Air				Air				Air		
	vols. per cent		*mm.* Hg		*vols. per cent*		*mm.* Hg		*vols. per cent*		*mm.* Hg		*vols. per cent*		*mm.* Hg
0	46.4			0	48.0			0	46.5			0	46.1		
20	46.0	46.2	30.0	20	46.9	47.4	31.0	20	43.5	45.0	38.0	20	44.1	45.1	26.0
30	High CO_2			30	High CO_2			30	High CO_2			30	High CO_2		
35	42.6			35	43.6			35	41.4			35	42.2		
48	41.5			48	42.9			48	42.3			48	42.7		
62	40.7			62	43.1			62	42.1			62	42.0		
74	41.2	41.5	90.0	74	43.7	43.3	92.0	74	41.7	41.8	87.0	74	42.5	42.3	84.0

TABLE 6

The transfer of bicarbonate between the blood and tissues of dogs, caused by diminishing the carbon dioxide concentration in the lungs

EXPERIMENT 44				
Weight 12.2 kgm.				
Minutes	CO_2 at 40 mm.	Averages	Alveolar CO_2	Blood pressure
	Air			
	vols. per cent		*mm. Hg*	*mm. Hg*
0	46.6			170
		46.6	46.0	
25	Overventilation			
48	44.2			144
63	45.1			
73	45.5			
83	46.1	45.2	19.0	150
85	Air			
90				166

EXPERIMENT 45				
Weight 11.4 kgm.				
Minutes	CO_2 at 40 mm.	Averages	Alveolar CO_2	Blood pressure
	Air			
	vols. per cent		*mm. Hg*	*mm. Hg*
0	41.4			
24	41.3	41.3	46.0	150
30	Overventilation			
37	43.1			100
50	42.9			110
60	42.6			
75	42.2	42.7	12.0	100
76	Air			
81	41.3			132
95	41.7			120
110	42.2	41.7		120

EXPERIMENT 46				
Weight 12.2 kgm.				
Minutes	CO_2 at 40 mm.	Averages	Alveolar CO_2	Blood pressure
	Air			
	vols. per cent		*mm. Hg*	*mm. Hg*
0	41.4			
32	40.9	41.1	35.0	168
40	Overventilation			
60	40.7			95
70	41.4			
80	42.3			92
95	41.4	41.4	8.0	84
98	Air			
115	39.4			157
135	42.9			
		41.1		154

EXPERIMENT 47*				
Weight 9 kgm.				
Minutes	CO_2 at 40 mm.	Averages	Alveolar CO_2	Blood pressure
	Air			
	vols. per cent		*mm. Hg*	*mm. Hg*
0	48.2			130
25	47.4	47.8	46.0	124
45	Overventilation			
60	45.7			70
69	45.6			
85	45.1			85
96	45.9	45.5	8.0	92
100	Air			
110	43.3			126
125	44.9			
		44.1		124

EXPERIMENT 48*				
Weight 8.5 kgm.				
Minutes	CO_2 at 40 mm.	Averages	Alveolar CO_2	Blood pressure
	Air			
	vols. per cent		*mm. Hg*	*mm. Hg*
0	40.4			
27	41.5	40.9	34.0	144
33	Overventilation			
41	43.8			109
55	41.7			
64	41.9			96
77	40.5	42.0	8.0	100
78	Air			
87	38.4			132
107	40.9			
		39.6		135

EXPERIMENT 56				
Weight 9.9 kgm.				
Minutes	CO_2 at 40 mm.	Averages	Alveolar CO_2	Blood pressure
	Air			
	vols. per cent		*mm. Hg*	*mm. Hg*
0	47.9			
24	47.2	47.5	46.0	161
28	Overventilation			
38	48.2			62
48	45.8			
58	46.3			70
73	45.7	46.5	9.0	86
75	Air			
86	46.0			140
103	47.4			
		46.7		142

* Positive pressure pump.

of carbon dioxide to 43.6 volumes per cent. After breathing the carbon dioxide mixture for 43 minutes, room air was substituted and the bicarbonate level of the blood rose again to 45.8 volumes per cent carbon dioxide, only 0.4 volume per cent less than the original level.

In summarizing the average results on dogs as given in table 4, it is found that during the inhalation of a gas mixture containing 11 per cent carbon dioxide (for a period of 40 minutes), the alveolar carbon dioxide tension was raised from 43 to 88 mm. of mercury, and the bicarbonate level of the blood fell from 44.1 to 42.0 volumes per cent of carbon dioxide. The level fell in individual cases from 0.3 to 3.3 volumes per cent carbon dioxide and not a single instance of a rise was observed. During the recovery period on air, immediately following the inhalation of the carbon dioxide gas mixture, the bicarbonate level returned to 44.6 volumes per cent carbon dioxide.

The effect which high alveolar carbon dioxide tensions have upon the bicarbonate level of cats' blood is given in table 5. The level falls from 45.5 to 41.3 volumes per cent carbon dioxide during the inhalation of the carbon dioxide gas mixture, a departure from the normal level which is just twice as great as in the case of dogs.

The effect of lowering the alveolar carbon dioxide tension upon the bicarbonate level of blood. The data are shown in table 6. Only dogs were used. The effect upon the bicarbonate level of reducing the alveolar carbon dioxide tension to 8 mm. by over-ventilation was negligible. In some cases the level was slightly raised and in other cases slightly depressed. The approximation to the normal bicarbonate level which followed the discontinuance of over-ventilation was not as close as that which followed the discontinuance of the carbon dioxide inhalation.

The fall in the blood pressure caused by over-ventilation is undoubtedly due to the lowered carbon dioxide tension throughout the body, as shown by Dale and Evans (1922). An alveolar carbon dioxide tension of about 8 mm. is probably the limit which is consistent with a steady state during over-ventilation and with a normal recovery after the return to natural breathing. The apnea which followed the discontinuance of the artificial respiration was only of short duration, and an approximation to the normal blood pressure followed immediately. It is very apparent that over-ventilation within the limits set by our experiments is in no way prejudicial to a complete and instant return to the normal condition after natural breathing has been reëstablished.

Over-ventilation was administered by the negative pressure method (Shaw and Drinker, 1929) in experiments 44, 45, 46, and 56, and by the positive pressure method through a tracheal cannula in experiments 47 and 48. Since the former method of over-ventilation is free from any interference with the pulmonary circulation or venous return to the heart,

we had hoped by its application to attain low levels of alveolar carbon dioxide unattended by the marked fall in blood pressure which characterizes artificial ventilation by intratracheal insufflation. The blood pressure was affected to the same extent by both methods of over-ventilation. This affords further evidence that the fatal effects of excessive over-ventilation are probably due to lowering of the carbon dioxide tension of the body rather than to the mechanical interference with the circulation.

Discussion. Our work is wholly at variance with that of Henderson and Haggard (1918a). They found that when the alveolar carbon dioxide tension was increased from about 40 mm. tension to 52 mm., as a result of the depressed breathing following morphine administration, the bicarbonate level of the blood was increased by about 12 volumes per cent. During the rebreathing of a carbon dioxide gas mixture from a gas bag, the bicarbonate level of the blood was raised 13 volumes per cent when the concentration of carbon dioxide in the respired air reached 10.7 per cent. In these last experiments the alveolar carbon dioxide tension was probably about 90 mm. and was comparable to the alveolar tension of carbon dioxide attained in our own experiments. When dogs were subjected to passive over-ventilation, on the other hand, Henderson and Haggard (1918b) found that the bicarbonate level was lowered by 12 to 22 volumes per cent of carbon dioxide.

In order to ascertain the possible effect which the barbital sodium might have upon the change in the bicarbonate level caused by the inhalation of carbon dioxide, we performed two experiments upon dogs without the use of barbital sodium. The gas mixture containing 8.3 per cent carbon dioxide was administered from a head mask, and the blood samples were withdrawn from the femoral artery, which had been cannulated under local anesthesia. In one case the bicarbonate level fell 3 volumes per cent and in the other case fell 1.6 volumes per cent following the inhalation of the gas mixture. These results are comparable to those given in table 4. It was also found that the bicarbonate level of blood drawn from the jugular vein of normal intact dogs breathing room air was identical to that of the blood drawn from dogs 3 hours after the intravenous injection of barbital sodium. These experiments make it clear that the bicarbonate level of blood is in no way affected by an intravenous injection of barbital sodium after time has been allowed for equilibrium conditions to become established.

Carbon dioxide absorption curves were constructed for blood taken from dogs while breathing air and compared with the curves for blood taken during the inhalation of the gas mixture containing 10 per cent carbon dioxide. The data are given in table 7. Though the absorption curves of blood taken during the inhalation of carbon dioxide are not in all cases parallel to those made from normal blood, the former are, in every case

and at all points, lower than the latter. Though the buffering qualities of the blood subjected in vivo to high tensions of carbon dioxide appear to have been somewhat altered from the normal, in no instance do the curves cross, thereby converting an apparent fall of the bicarbonate level as estimated at a given carbon dioxide tension into a rise when estimated at a different tension. There is a lowering of the bicarbonate level of blood taken during the inhalation of carbon dioxide at all carbon dioxide tensions.

The readiness with which sodium bicarbonate passes from the blood into the tissue fluid, and presumably in the reverse direction under appro-

TABLE 7

The effect of high alveolar carbon dioxide tensions upon the carbon dioxide absorption curve of dogs' blood

EXPERIMENT	20 MM.			40 MM.			60 MM.		
	Air	CO_2	Difference	Air	CO_2	Difference	Air	CO_2	Difference
20	30.3	26.0	4.3	42.2	39.3	2.9	50.4	47.1	3.3
21	29.1	29.4	0.3	43.5	41.9	1.6	53.4	50.5	2.9
23	31.0	26.0	5.0	42.3	39.4	2.9	48.0	46.0	2.0
24	26.1	23.0	3.1	34.0	32.5	1.5	42.0	36.6	5.4

TABLE 8

The per cent of sodium bicarbonate which is retained by the plasma 30 minutes after its injection intravenously

EXPERIMENT	PLASMA VOLUME	VOLUME CARBON DIOXIDE INJECTED	VOLUME CARBON DIOXIDE RETAINED BY PLASMA AFTER 30 MINUTES	PER CENT RETAINED
	cc.	*cc.*	*cc.*	*per cent*
1	107	267	23.3	8.7
2	103	280	25.5	9.1
3	103	259	31.0	12.0
4	128	292	21.8	7.5
5	128	289	26.9	9.3

priate conditions, can be shown by a very simple experiment. About 50 cc. of an 0.268 molar solution of sodium bicarbonate were injected slowly into the femoral vein of a cat. Except for the small quantity of sodium bicarbonate accumulating in the pelvis of the kidney, escape via the kidney was prevented by the ligation of the ureters. At 15-minute intervals blood samples were taken under oil and the plasma, which was allowed to separate from the whole blood while resting over mercury in a blood collecting tube, was analyzed for carbon dioxide content. The total volume of plasma in the cat was calculated on the basis that the blood volume was 5.5 per cent of the body weight and the plasma 65 per cent of the whole blood. The amount of bicarbonate which was retained by the plasma

at the end of a given time following the injection was determined by the departure from the normal carbon dioxide content of the blood. Table 8 gives the data for five experiments. Speaking in terms of averages it shows that, following an injection of sodium bicarbonate solution carrying 277 cc. of carbon dioxide, only 25.7 cc., or 9.3 per cent of the original injection, were left in the plasma at the end of 30 minutes.

It follows from these considerations that whenever the bicarbonate level of the plasma is raised above that of the tissue fluids, bicarbonate will leave the blood; and conversely, when the bicarbonate level of the plasma is depressed below that of the tissue fluids, bicarbonate will flow into the blood. In order to eliminate confusion we may safely overlook the slight difference which may exist under conditions of equilibrium between the bicarbonate level of the plasma and tissue fluids caused by the Donnan equilibrium.

The bicarbonate of the plasma is due to the reaction of carbonic acid with the alkali salts of the protein and with the chlorides according to the following equations:

$$(1) \qquad H_2CO_3 + \text{Na Proteinate} = NaHCO_3 + \text{H Proteinate}$$

$$(2) \qquad H_2CO_3 + NaCl = NaHCO_3 + HCl$$

In equation 1, sodium proteinate is used to indicate the alkali salts of the plasma proteins, and the hydrogen proteinate the acid proteins. The hydrochloric acid in equation 2 will pass into the blood cells leaving one molecule of sodium bicarbonate in the plasma for every molecule of hydrochloric acid bound by the alkali buffers of the blood cells. The equilibria as written will shift from left to right with an increase of carbonic acid and cause an increase of bicarbonate. As the carbonic acid decreases, the equilibria will shift from right to left and the bicarbonate will be diminished.

In explanation of the migration of bicarbonate out of the blood we offer the following hypothesis. When the carbon dioxide tension in the lungs is raised, the bicarbonate content of the blood is increased, followed immediately by an increase in the bicarbonate content of the tissue fluids. If the carbon dioxide combining capacity of the tissue fluids were exactly equal to that of the blood then the bicarbonate level in the two systems of fluid would remain equal. Under these conditions no transfer of bicarbonate between the blood and tissue fluids would take place. On the other hand, if the carbon dioxide combining capacity of the blood were greater than that of the tissue fluids, then an increase in the carbon dioxide tension of the lungs would cause the formation of more bicarbonate in the blood than in the tissue fluids, and the bicarbonate level in the blood would rise above the level attained in the tissue fluids. Under these conditions bicarbonate would move out of the blood into the tissue fluids until equilibrium is again established.

The work of Shaw and Messer (1930) upon the carbon dioxide combining

capacity of the tissue fluids gives support to the above hypothesis. They made cats breathe a gas mixture containing about 11 per cent carbon dioxide for a sufficient time to bring the entire body into equilibrium with the increased carbon dioxide tension of the alveolar air. Outdoor air was then substituted for the gas mixture and the expired air was collected during the period of desaturation. The carbon dioxide that was retained by the body, and subsequently eliminated in response to a return of the normal alveolar carbon dioxide tension, could then be evaluated by subtracting the metabolic carbon dioxide from the total carbon dioxide. Since the carbon dioxide retained by the whole body is held in the fluids of the body, the carbon dioxide taken up by the tissue fluids is equal to the carbon dioxide retained by the whole body minus the carbon dioxide taken up by the blood. Shaw and Messer found that when the carbon dioxide tension was raised from 30 mm. to 90 mm. of mercury, 1 kilo of cats' blood combined with 3.2 cc. of carbon dioxide per millimeter of carbon dioxide tension difference, and that 1 kilo of tissue fluid combined with 2.5 cc. per millimeter of carbon dioxide tension difference. The tissue fluids, therefore, have a carbon dioxide combining capacity which is 78 per cent as great as that of blood.

Since the buffer value of the tissue fluids of the cat is only 78 per cent of the blood capacity, it follows that there will be 22 per cent less bicarbonate formed in the tissue fluid than in the blood, following an increased carbon dioxide tension in the lungs. As a consequence the blood should lose 22 per cent of its bicarbonate in order that equilibrium may be established between the two systems of fluid. The bicarbonate level of the tissue fluid will not be appreciably affected by this transfer since the volume of the tissue fluid is at least ten times as great as the volume of blood, so that approximately nine-tenths of the adjustment takes place in the blood.

In our experiments on cats, the alveolar carbon dioxide tension was raised from 33 mm. to 90 mm., so that the blood absorbed about 18.2 volumes per cent of carbon dioxide (57×0.32 volumes per cent). We have seen that about 4.2 volumes per cent of carbon dioxide were lost. This is equivalent to a 23 per cent $\left(\frac{4.2}{18.2}\right)$ loss of the blood bicarbonate. Thus the estimated loss of bicarbonate (22 per cent) from the blood, based upon the relative buffering value of blood and tissue fluid, is in striking agreement with the observed loss (23 per cent).

When there is no migration of bicarbonate into or out of the blood we may assume that the buffer values of the blood and tissue fluids are equal. When there is a migration of bicarbonate it signifies that the buffer value of the blood is either greater or less than that of the tissue fluids, and that bicarbonate is moving in the direction of its lowest concentration. In the

case of the dog, the migration of bicarbonate takes place very slightly in both directions when the carbon dioxide tension has been lowered, indicating that at tensions below normal the carbon dioxide absorption curve of the tissue fluids is essentially coincident with that of the blood. When the carbon dioxide tension has been raised, bicarbonate migrates out of the blood, indicating that at some tension above the normal the carbon dioxide absorption curve of the tissue fluids falls below the blood curve and becomes somewhat flatter.

Following a rise in the carbon dioxide tension of the lungs, the migration of bicarbonate into the tissue fluids must take place very rapidly. Since the first sample of blood was taken 5 minutes after the inhalation of carbon dioxide commenced, and was in substantial agreement with samples taken during the next 35 minutes, it is certain that the transfer of bicarbonate was complete in less than 5 minutes.

One might expect that the immediate effect of increasing the bicarbonate concentration of the blood before the concentration in the tissue fluids had been increased, as must be the case when carbon dioxide is inspired, would be a temporary but very marked overflow of bicarbonate into the tissue fluids with a subsequent return of bicarbonate to the blood as the reaction takes place throughout the body. Shaw and Messer (1930) have shown that the body as a whole takes about 90 minutes to come into equilibrium with changes in the carbon dioxide tension of the blood. That equilibrium between the bicarbonate of the blood and the body fluids is established so promptly and is not conditioned by the reaction time of the entire body, is probably due to the fact that carbon dioxide diffuses through tissues more rapidly than bicarbonate, and therefore the bicarbonate concentration of the tissue fluids in immediate contact with the blood is raised to its equilibrium value before the bicarbonate from the blood has passed through the capillary walls. Though it may require approximately 90 minutes for the bicarbonate concentration of the tissue fluid of the entire body to attain its maximum concentration following an increased carbon dioxide tension in the lungs, the concentration of the bicarbonate in the tissue fluid in direct contact with the capillary walls will attain its maximum concentration almost immediately, and thereby establish the equilibrium level for the bicarbonate of the blood.

SUMMARY

1. When the alveolar carbon dioxide tension was raised to about 88 mm. by the inhalation of air enriched with carbon dioxide, the carbon dioxide combining capacity of the blood was lowered by 2.1 volumes per cent in dogs and by 4.2 volumes per cent in cats, as a result of the migration of bicarbonate into the tissue fluid. The time required to bring about equilibrium conditions was less than 5 minutes.

2. When the alveolar carbon dioxide tension of dogs was lowered to about 8 mm., the carbon dioxide combining capacity of the blood was increased in some cases by the migration of bicarbonate into the blood, and was diminished in other cases by the migration of bicarbonate out of the blood, the average change being 1.4 volumes per cent carbon dioxide.

3. An hypothesis, based upon the relative carbon dioxide combining capacity of the blood and the tissue fluids, is offered to explain the transfer of bicarbonate between the blood and the tissues.

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THE EFFECT OF THE INJECTION OF HYPOPHYSEAL EXTRACT IN ADVANCED LACTATION

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During the past two years our conception of the stimuli which are responsible for mammary development has undergone a profound alteration. It has been held as a result of the pioneer work in this field that the stimulus is due entirely to the action of the corpus luteum. But the evidence although it seemed overwhelming, was circumstantial in character and under the fire of recent experimental evidence the interpretation, justifiable at the time, placed upon the results of these pioneers has been thrown open to doubt. In 1929 Grueter and Stricker announced that they had been able to induce full mammary development in the ovariectomised mature rabbit by the injection of an alkaline extract of the anterior pituitary. They found further that the injections were without effect in the case of immature animals, and from an examination of the ovaries of their rabbits concluded that the mammary gland requires a preliminary sensitization by the action of the rabbit's own corpus luteum. Corner (1930), using an alkaline extract of sheeps' whole pituitary, obtained full mammary development in mature virgin rabbits which had been ovariectomised. As examination of the ovaries of the operated animals showed that ovulation had never occurred, it is evident that previous action of the corpus luteum is unnecessary. In addition Corner found that the corpus luteum extract from the sow which would maintain pregnancy in the absence of the rabbit's ovaries is without effect upon the mammary gland when administered to the non-pregnant doe.

Later, Asdell, using the same neutralised alkaline extract of sheeps' whole pituitary supplied by Parke, Davis & Company, was able to confirm the production of full mammary development similar to that of pregnancy. His ovariectomised rabbits were just mature, but careful examination of the ovaries showed that ovulation and consequent formation of corpora lutea had never occurred. It was also found that the potent extracts are without effect in the immature rabbit. It is evident, therefore, that some growth or sensitization is needed before the hypophyseal preparation can become effective. The results were extended to

a goat as daily subcutaneous injection of 10 cc. of the extract (3.35 cc. = 1 gm. whole pituitary) into a young female goat which, so far as was known, had not been in heat resulted in sufficient mammary development to be recognized by a slight increase in the size of the udder while it was possible to express a few drops of milk from the teats. This had been impossible in repeated trials before the injections. The possible stimulating action of handling the gland was guarded against as the udder was not touched for the fortnight during which injections were in progress. Injection of 40 cc. daily into a mature goat, whose lactation period was nearly spent, failed. The extract as prepared at present is very toxic and the goat became emaciated and refused to feed. The milk yield declined. A preliminary report of these experiments has been made (Asdell, 1931).

As the preliminary work had shown that the extract is probably effective in the goat, an attempt was made this year to determine whether continued administration of the extract to the mature lactating goat would prevent the normal decline in lactation by maintaining the growth of the mammary gland at its normal maximum or beyond it. Messrs. Parke, Davis Company again generously supplied the considerable quantity of extract which was necessary for this work. Each batch was freshly made and despatched by express at regular intervals. It was kept in an ice chamber during the experiment and tested by injection into ovariectomised rabbits. It proved to be potent. The method of preparation and strength were as before. The dose used to test the effect of the extract on rabbits was 3 cc. per day for 8 days. Three goats were used in the main experiment and the injections arranged as follows:

Goat D. Date of parturition, January 29, yield at time of experiment, June 1, 1700 cc. daily. Injected subcutaneously with 10 cc. after the evening milking.

Goat B. Date of parturition, January 29; milk yield 1700 cc. Daily injection 20 cc.

Goat C. Date of parturition, February 4; milk yield 1600 cc. Daily injection 30 cc. from June 12 to July 2 when injection was dropped to 10 cc. daily as the goat began to show ill effects from the injections.

Three control goats A, E and F, dates of parturition February 24, January 29, and February 21 respectively, received no injections but were kept in the same room under similar conditions. All goats were milked at 8:30 a.m. and 4:00 p.m.

The injections commenced on June 12th and continued until July 24th.

Goat B was considerably older than the others and she soon refused to feed and became emaciated. Her milk yield dropped considerably and she was discarded. The toxicity of the extract is considerable and she evidently felt the ill effect. The yield of the other two goats increased for a period of 36 days but they then refused their feed and lost weight. The milk yield consequently declined rapidly. This ill effect was probably due to the accumulated toxicity of the extract. The use of a new batch

of extract three days after the decline in milk yield began did not improve the yield.

It is to be noted that the milk yields of the injected goats were increasing at the time when those of the controls showed a marked falling off. This is the more remarkable as it includes a period of exceptionally hot

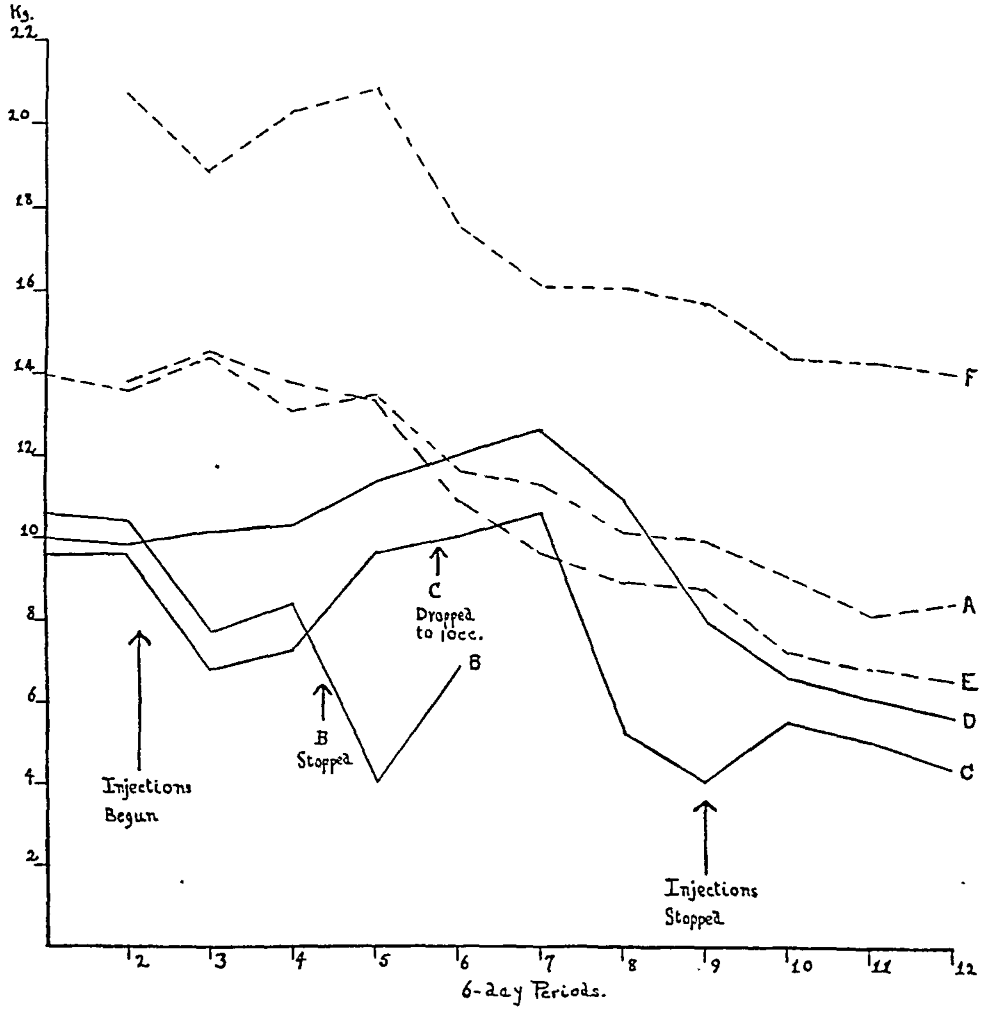

Fig. 1. Anterior hypothesis and milk yield in goats. Ordinates: milk yield in kilograms by 6-day periods. Abscissae: 6-day periods. Controls: broken lines. Injected goats: continuous lines. Daily injections: Goat D, 10 cc. Goat B, 20 cc. Goat C, 30 cc. at first, then 10 cc.

weather. In the graph, the daily milk yields have been added and the quantity yielded in six day periods reported. We feel that the conclusion is justifiable that the extract of anterior lobe is effective in preventing, for a time at any rate, the normal decline in milk yield with advancing lactation. Whether the gland can be built up further than is normal for

the animal, and whether the total milk yield for the lactation period can be increased remains to be determined in future work with a less toxic preparation.

SUMMARY

Repeated daily injections of alkaline extract from sheep hypophyses prevent, for a time at least, the normal decline of milk yield in goats with advancing lactation.

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RELATION OF PROLAN TO THE ANTERIOR HYPOPHYSEAL HORMONES[1]

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Aside from its value as a test for pregnancy, the discovery of a gonad-stimulating hormone in the urine of pregnant women by Aschheim and Zondek has acquainted us with a new fact of the deepest interest to those concerned with the physiology of reproduction. The source of this hormone is still unknown to us, though it has been generally assumed that it comes from the hypophysis. Its effect on the ovary of an infantile animal resembles strikingly the effect produced by "implants" of anterior hypophyseal tissue and of no other tissue known to us save the placenta. It was hence natural to assume that this urinary constituent originates in the hypophysis.

As this laboratory has been concerned for some years with hypophyseal hormones, we early instituted comparisons between the gonad-stimulating hormone from the urine and that which can be extracted from the hypophysis.[2] There are some striking differences between these substances. When prolan is administered in increasing doses, the response of the ovary corresponds to the dosage only up to a certain limit. When doses are given exceeding this limit, no matter what the dose or how often it is administered, the effect on the ovaries is practically the same. If, on the other hand, one gives increasing dosage either in the form of implants of hypophysis, or of extracts of hypophysis, the weights of the ovaries produced increase with the dosage. As an example of the effect of increase in implant dosage we cite an experiment from a previous paper.[3]

A single implantation of half of a rat's anterior lobe caused fifty per cent of the animals to mature and the average ovary weight was 21 mgm. With all higher im-

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[1] Aided by grants from the Committee for Research in Problems of Sex of the National Research Council, and from the Rockefeller Foundation. These funds have been generously augmented by the Board of Research and the College of Agriculture of this University.

[2] For convenience in this paper we will use Zondek's term "prolan" to designate the hormone from urine. By "gonad-stimulating" hormone we do not necessarily imply a single substance. We do not wish to discuss the problem of multiplicity of gonad-stimulating hormones in the hypophysis at this time.

[3] Evans, H. M. and M. E. Simpson. This Journal, 1929, lxxxix, 381.

plant "dosage" practically all animals matured in 100 hours and the ovary weights varied with the dose level as follows: a dose of one anterior lobe gave ovaries weighing 68 mgm.; 4 lobes gave ovaries weighing 105 mgm.

In the following chart (fig. 1) the effect of increasing the dosage of extracts from the anterior hypophysis is contrasted with the effect of increasing the dosage of prolan. The response of the ovary of the immature test animal to different dose levels of these substances was measured by the weight of ovaries produced within 100 hours. It can be seen that

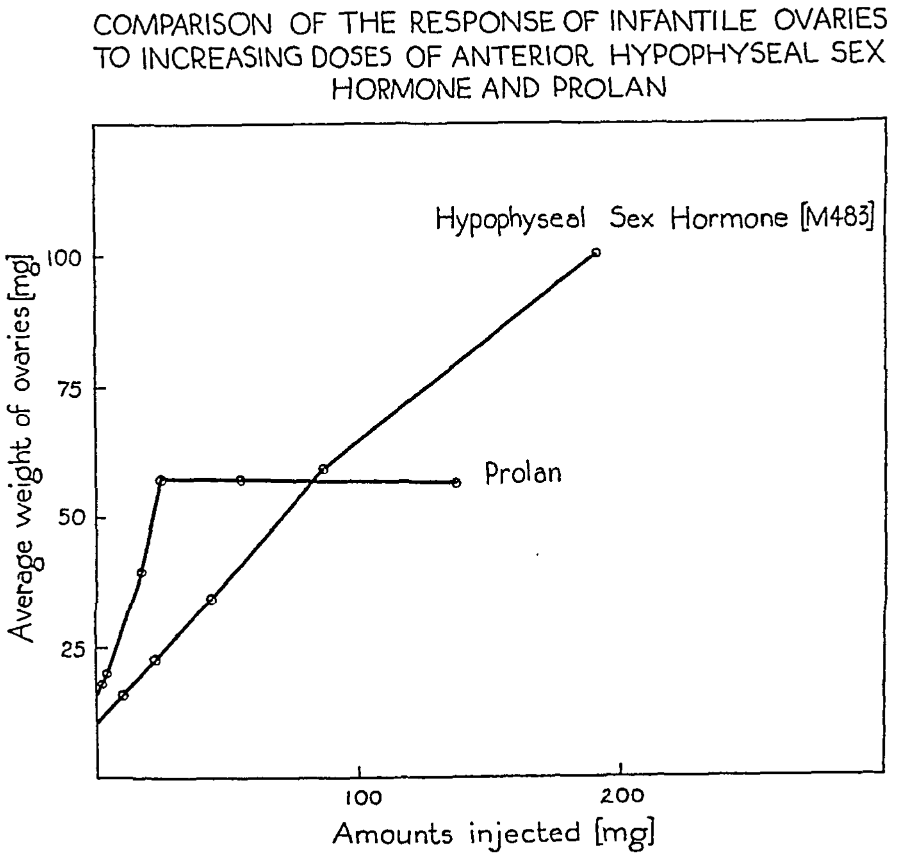

Fig. 1

in the case of prolan administration, the curve in which ovary weight is plotted against dose level quickly reaches a plateau and that a further fivefold or even tenfold increase in dose does not cause a further increase in ovary weight. In the case of administration of the anterior hypophyseal gonad-stimulating hormone, the curve expressing the relation between dose and ovary weight continues a steep ascent as higher dose levels are reached. It has been possible by injection of hypophyseal sex hormone to provoke ovaries weighing up to 190 mgm. within 100 hours. On the other

hand, it is seldom possible even with 200 or more units[4] of prolan to stimulate the production of ovaries weighing over 70 mgm. in this time interval. In cases of administration of other preparations of the hypophyseal sex hormone we have found the ascent of the curve to be even steeper than in the case recorded here. All of our extracts of the gonad-stimulating hormone from the hypophysis itself whether crude or purified were alike in showing this type of curve.

Perhaps the most important reason for assuming that prolan and the hypophyseal gonad-stimulating hormone are different is the relative inefficacy of prolan in hypophysectomized animals as reported in the preceding paper of this series. In contrast to these findings it has been shown by Smith, Reichert and others that the infantile reproductive systems of such hypophysectomized animals can be stimulated to complete development and function by the administration of anterior hypophyseal material.[5]

Briefly then, though prolan and anterior hypophyseal hormone appear to have the same qualitative effect on the ovary of the normal infantile animal, they are dissimilar in that prolan is embarassed in its action in the hypophysectomized animal and is limited in the effects it can provoke in the normal infantile animal. On the other hand the gonad-stimulating anterior hypophyseal hormone is effective in the hypophysectomized animal, and does not suffer the curious limitation of its effect on the ovaries of the normal immature animal. We must assume, it would appear, that the hypophysis itself plays a rôle in the action of prolan. Since prolan appears to have the same qualitative effect on the ovary as has the hypophyseal hormone, it would seem that we must think of prolan either as *provoking the production of the gonad-stimulating hormone of the hypophysis, or as converting some inactive component in the hypophysis into an active substance.* The limited effect of prolan on the normal infantile ovary would be clear for it could be considered as a direct consequence of low content or low production of the mother substance in the hypophysis ofthe immature animal. Even in the sexually mature animal the amount of active substance that can be demonstrated by the implantation method or by extraction is surprisingly low, especially in beef.

Since we can extract directly from the hypophysis the gonad-stimulating hormone, we must assume, if the previously expressed theory is correct, either that this amount of hormone had been previously activated within

[4] The unit of prolan to which reference is made is the minimum dose which administered in three daily subcutaneous doses to immature rats produces in 96 hours the development of ovaries containing a corpus or corpora. Only one of the three test animals needs respond.

[5] Smith, P. E. The disabilities caused by hypophysectomy and their repair. Journ. Amer. Med. Assoc., 1927, lxxxviii, 158. Reichert, F. L., The results of replacement therapy in an hypophysectomized puppy: four months of treatment with daily pituitary heterotransplants. Endocrinol., 1928, xii, 451.

the hypophysis by a prolan-like substance; or that it had been activated by a substance freed during preparation, or finally, that the inert substance or prohormone had been activated at the site of injection. The assumption that an activator is present in the hypophysis itself seems more probable to us.

A direct proof of this theory of the activation of an inert substance in the hypophysis would be furnished if by adding the activator to a given preparation from the hypophysis we could produce effects not secured from the hypophyseal preparation alone. The experimental confirmation of the theory was surprisingly satisfactory. The combination of prolan and hypophyseal extract gave a far greater effect than could be obtained from the administration of either component alone or than could be expected on the basis of an additive effect.

Experimental. The procedure was as follows: groups of three animals 24–26 days of age were injected daily, subcutaneously, for three successive days (Monday, Tuesday, Wednesday) with 1 cc. doses of the different aqueous preparations. The animals were observed twice daily after the 50th hour (Wednesday) for rupture of the vaginal membrane. After 96 hours, i.e., on the fourth day (Friday), the animals were sacrificed and the genital tract was examined under a binocular microscope. The ovaries were dissected free of bursa and oviduct and weighed on an analytical balance to an accuracy of 1 mgm. The size and vascularity of the genital systems and in particular the size and number of follicles and corpora and the presence of blood points were noted. The ovaries were sectioned when occasion demanded.

The methods of preparation of extracts from hypophysis and urine will be reported in detail in another paper. We are concerned in this paper with more purely biological investigations. The source of the hormone from the urine of pregnant women used by us was a crude prolan kindly furnished to us by the I. G. (Elberfeld)[6]. The crude prolan powder was extracted with acidulated water and poured into alcohol. The precipitate was washed with alcohol and ether and quickly dried in vacuo. The minimal potent dose of this powder when redissolved in water was 1.5 mgm. (0.5 mgm. daily for 3 days). Beef hypophyses were the source of the anterior lobes used by us in the preparation of the hypophyseal extracts reported here. Stable dry powders were prepared by acetone precipitation and were used in most of the experiments reported in this paper. Unless otherwise stated, they contained both the gonad-stimulating and the growth hormone. Other more purified preparations in which growth and sex hormones had been separated were also used and will be indicated in the appropriate place in the text and tables. All preparations were brought to neutrality (pH 7.4) before injection.

[6] In particular we wish to thank Dr. Werner Schulemann and Dr. Fritz Lacquer.

TABLE 1

Effects on the ovary of the immature rat of prolan and hypophyseal hormones given separately and in combination

	DESIGNATION OF EXPERIMENT																
	486	502	509	307	332	308	328	329	456	502	459	429	431	430	419	426	421
Dose* prolan, mgm.	54.2	0	54.2 (#486)	136.3	0	136.3 (#307)	54.5	54.5 (#328)	54.5	0	54.5 (#456)	136.3	0	136.3 (#429)	13.6	0	13.6 (#419)
Dose A. H. H., mgm.	0	65.0	65.0 (#502)	0	65.0	65.0 (#332)	0	65.0 (#332)	0	65.0	65.0 (#502)	0	65.0	65.0 (#431)	0	65.0	65.0 (#426)
Average weight of ovaries, mgm.	62.0	21.3	156.3	59.7	22.7	131.3	59.3	136.3	60.0	21.3	117.0	56.7	21.3	117.7	37.3	26.7	74.7
Activation			151%			120%		121%			95%			107%			100%

* By "dose" we mean the weight in mgm. of dried material dissolved in 3 cc. of water and administered to each animal in 1 cc. doses for 3 days.

Examples of activation of hypophyseal preparations by prolan. In table 1 we present examples of the effect on the immature ovary of combining prolan with hypophyseal preparations. Given amounts of prolan and hypophyseal preparations were administered, separately and after combination. The combination was made in vitro. The total volume was equal to that of the individual constituents when injected alone. The dose levels of hypophyseal preparations were chosen carefully so that they were barely able to provoke precocious maturity (with corpora formation) or fell just below the minimum dose level. The prolan preparations were usually administered at a dose level at which they already provoked the maximum effect possible with prolan. They stimulated numerous corpora lutea, large follicles and follicular cysts. It can be seen in the table that the injection of the combination of these constituents, administered at these dose levels, stimulated the development of ovaries weighing far more than the ovaries provoked by the individual constituents alone—also larger ovaries than could be expected from any additive effect resulting from the combination of the two constituents. The ovaries stimulated were usually characterized by great numbers of corpora though large follicles and follicular cysts were often present.

The percentage activation was calculated as the relation between ovary weight stimulated by prolan and the increased weight due to the combination. The ovary weight of the hypophysis-injected rats was neglected in our calculation of activation because the weight of these ovaries was seldom more than the weight of infantile ovaries.

Specificity of the activation. We next wish to give the evidence for considering that the activation of hypophyseal preparations is a specific reaction. We tested the specificity of this reaction in three ways: first, we combined prolan with active and inactivated hypophyseal hormone; secondly, we combined prolan with extracts from other organs prepared in a manner identical with that used in preparing hypophyseal extracts; thirdly, we substituted for prolan extracts from tissues prepared in the same way prolan is prepared.

The hypophyseal hormone was inactivated by being heated for ten minutes in vigorously boiling water, or for 30 minutes at 70–80°C. The hypophyseal hormone heated for 30 minutes at 70–80°C., when administered in combination with prolan, no longer produced large ovaries but only those of the weight provoked by prolan alone.[7] The shorter treatment at 100°C. apparently did not completely inactivate the hypophyseal component so that the result of combination of the heated product with prolan while less than that in cases where active hormone was used (see

[7] Since the visible physical properties of the hypophyseal hormone preparation when heated to 70–80°C. were unchanged, the probability that the activation phenomenon is due to some physical and hence non-specific effect is excluded.

table 2 A) was still greater than the effect of administration of prolan alone.

The second method of testing the specificity of the activation was as follows: calf and pig liver were extracted and treated in a manner similar

TABLE 2A

Specificity of the activation effect obtained by combining prolan and hypophyseal hormones; (A, Effect of the destruction of the specific substances by heat)

	DESIGNATION OF EXPERIMENT								
	328	332	329	334	330	307	308	310	309
Dose prolan, mgm.	54.5 (#307)	0	54.5 (#307)	0	54 5 (#307)	136.3	136.3 (#307)	0	136.3 (#307)
Dose A. H. H., mgm.	0	65.0 (Active solution)	65.0 (Active solution #332)	65.0 (Inactivated 34′ at 70–80° C. #332)	65.0 (Inactivated 34′ at 70–80° C. #332)	0	65.0 (Active solution #332)	65.0 (Inactivated 10′ at 100° C. #332)	65.0 (Inactivated 10′ at 100° C. #332)
Average weight of ovaries, mgm.	59.3	22.7	136 3	13.7	62.3	59.7	131.3	16.7	79.0
Activation			121%		None		120%		Inconsiderable

TABLE 2B

Specificity of the activation effect obtained by combining prolan and hypophyseal hormones; (B, Substitution of a non-specific substance, liver, for the hypophyseal component)

	DESIGNATION OF EXPERIMENT						
	486	505	503	509 (control)	546	549	556
3 days prolan dosage, mgm.	54.5	0	54.5 (#486)	54.5 (#486)	54.5	0	54.5 (#546)
3 days liver dosage, mgm.	0	54.5	54.5 (#505)	65.0 (A. H. H. #502)	0	65.0	65.0 (#549)
Average weight of ovaries, mgm.	62.3	17.3	55.3	156.3	53.7	17.0	47.5
Activation			None	150%			None

to that used in making hypophyseal preparations. This product was administered separately and in combination with prolan as in the usual procedure. In table 2 B it can be seen that the potency of the prolan was not increased by combination with the liver preparations. But the

potency of the same prolan was increased when combined with a hypophyseal preparation. This is shown in the table for contrast.

It would seem from the above data that the hypophyseal component of our combination is certainly a specific one. We have endeavored to examine similarly the specificity of prolan. Since the activation we describe resembles a ferment reaction, we tried to substitute certain ferments in place of prolan. We chose liver as the source of the ferment partly because the liver is well known to be rich in these substances, but chiefly because we suspected that the reaction described in this paper might

TABLE 2C

Specificity of the activation effect obtained by combining prolan and hypophyseal hormones; (C, Substitution of a non-specific substance for the prolan)

	DESIGNATION OF EXPERIMENT								
	505	502	504	506	549	555	548	550	551
3 days dose liver, mgm........	54.5 (Pig)	0	54.5 (#505)	27.2 (#505)	54.5 (Calf)	0	54.5 (#549)	54.5 (#549)	54.5 (#549)
3 days dose A. H. H., mgm.......	0	65.0	65.0 (#502)	65.0 (#502)	0	65.0	65.0 (#555)	65.0 (#555) 1 hr. at 37°C.	65.0 (#555) 1 hr. at 37°C.
Average weight of ovaries, mgm.......	17.3	21.3	35.7	28.3	17.0	30.7	37.7	42.7	34.7
Activation..			69%	33%			Inconsiderable	39%	Inconsiderable

bear a relation to the arginine content of the hypophysis. In a later paper we will elaborate on the reasons which led to these deductions. In table 2 C we give some examples showing the effect of administering a liver powder in combination with hypophyseal extracts. Although there was a slight difference in the effects of administering the hypophyseal preparation alone and in combination with liver, the difference may not be significant. To establish such a nonspecific activation would naturally require much further biological and chemical investigation.

Quantitative relations. We have considered two possible mechanisms

by which the activation process may take place. Either a stoichiometric combination occurs between the two components or the conversion of a mother substance, a prohormone, is facilitated by a catalyst. In the first case we should expect the reaction to involve definite proportions of the reacting constituents. If the second supposition were true, we should expect the reaction to be more nearly independent of one of the constituents, namely, the catalyst. We tried to decide between these possibilities by experiments in which we varied the amounts of each constituent, hypophysis and prolan. When we administered constant amounts of prolan

TABLE 3

Effect on ovaries of immature rats of combining different amounts of hypophyseal preparations with a constant prolan

	DESIGNATION OF EXPERIMENT								
	375	220	446	450	328	332	329	333	331
3 days prolan dosage, mgm.	27.2	0	27.2 (#375)	27.2 (#375)	54.5	0	54.5 (#328)	0	54.5 (#328)
3 days A. H. H. dosage, mgm.	0	112.5 (sex free growth hormone)	45.0 (#220)	22.5 (#220)	0	65.0	65.0 (#332)	6.5 (#332)	6.5 (#332)
Average weight of ovaries, mgm.	32.7	21.0	85.1	53.0	59.3	22.7	136.3	17.7	60.3
Activation			160%	62%			121%		None

with decreasing amounts of hypophyseal hormone the ovarian response diminished rapidly (table 3). When on the other hand the hypophyseal component was kept constant and the amounts of prolan were varied the activation values were quite constant after a certain concentration of prolan was reached. The results speak rather for the catalytic theory. When smaller amounts of prolan are used in the reaction there is a considerable decrease in the percentage activation as well as lower absolute values for the ovary weights (table 4).

Constancy of the activation reaction. In tables 5 and 6 we have given the results of several dozen experiments made in an effort to see whether

Effect on the ovary of he immature rat of the co

	307	332	308	328	329	429	431	430	3
3 days dose prolan, mgm..........	136.3	0	136.3 (#307)	54.5 (#307)	54.5 (#307)	136.3	0	136.3 (#429)	54 (#
3 days dose A. H. H., mgm........	0	65.0	65.0 (#332)	0	65.0 (#332)	0	65.0	65.0 (#431)	
Average weight of ovaries, mgm.....	59.7	22.7	131.3	59.3	136.3	56.7	21.3	117.7	52
Activation..........................			120%		121%			107%	

the activation reaction was one which could be duplicated quantitatively. This did not prove to be the case. In table 5 are summarized all results from experiments in which samples from the same prolan preparation were combined with a variety of hypophyseal preparations. In table 6 experiments are cited in which samples from a single acetone powder are combined with different prolan preparations. As can be seen from average

TABLE 5

Effect on the ovary of the immature rat of combinations of different hypophyseal preparations with the same prolan

	DESIGNATION OF EXPERIMENT							
	509	459	374	547	581	376	382	384
Dose prolan #108, mgm...	54.5	54.5	54.5	54.5	54.5	27.2	27.2	27.2
Dose A. H. H., mgm..........	65.0 (#120)	65.0 (#120)	65.0 (#107)	65.0 (#16)	65.0 (#120)	65.0 (#107)	65.0 (#97)	65.0 (#93)
Average weight of ovaries, mgm..........	156.3	117.0	96.0	88.5	110.0	63.7	63.0	48.3
Activation......	150%	95%	85%	65%	77%	95%	84%	48%

ovary weights and from percentages of activation, the results in both sets of experiments varied very considerably. We are aware of the fact that much of this variability can be traced to the small number (three) of animals in our experimental groups.

Component in the hypophysis activated. We have been able to resolve the crude acetone powders of hypophyseal substance employed in these

of prolan with a constant hypophyseal preparation

OF EXPERIMENT

	378	379	380	427	428	418	420	419	421	422	424	423	425
)	13.6 (#429)	2.7 (#429)	2.7 (#429)	136 3	136.3 (#427)	27.2 (#427)	27.2 (#427)	13.1 (#427)	27.2	27.2	27.2 (#422)	13.1 (#422)	13.1 (#422)
	65.0 (#431)	0	65 0 (#431)	0	65 0 (#431)	0	65.0 (#431)	0	65.0 (#431)	0	65.0 (#431)	0	65.0 (#431)
	49.3	19.0	27.0	64.0	114.0	44 3	78.7	37.3	74.7	39.0	72.3	34.7	65.0
	53%		38%		78%		79%		100%		85%		91%

studies into chemically and physiologically different substances, into the growth hormone and the gonad-stimulating hormone. We have prepared from the acetone powder, growth hormone free of the gonad-stimulating hormone, also the gonad-stimulating hormone free of the growth promoting factor.[8] We were naturally interested to know whether one or both of these two components of the acetone powder was responsible for the activation. Combinations of prolan with the gonad-stimulating hormone of the hypophysis gave no activation whatever (table 7). On the other hand a marked activation resulted in most cases in which prolan was combined with purified growth fractions (table 8). We have proven that our growth hormone preparations are free of gonad-stimulating hormone but we can not naturally be equally certain that other physiologically active substances are absent. Therefore we can not be sure at present whether some unknown substance is concerned. Nevertheless good grounds exist for the belief that the activated substance is the growth hormone. In the first place our various growth preparations were prepared in radically different ways. Secondly, although not crystalline, they are quite pure as judged by the fact that we have found them to be almost free from serum proteins.[9] Further reason for considering that it is indeed the growth hormone itself which is the activated substance is furnished by the following facts. Slight changes in methods of preparation can be made, which though not radical ones, are sufficient to cause destruction of the growth hormone. These slight changes in procedure are also followed by failure of the activation reaction on combination with prolan.

Discussion. It is necessary to bear in mind the following facts which have been ascertained: Prolan does not easily affect the ovary of the

[8] The purification was tested in each case by the largest quantities that could be administered—the limiting factors being solubility and the amount the animal could tolerate.

[9] Mr. W. R. Lyons has performed precipitin tests on these preparations and has shown that no more than 2 per cent of the total protein is beef serum protein.

TABLE 6

Effect on the ovary of the immature rat of combination of different prolan preparations with the same hypophyseal preparation

	DESIGNATION OF EXPERIMENT												
	509	459	581	582	438	428	430	420	424	378	421	425	378
Dose prolan, mgm.	54.5 (#108)	54.5 (#108)	54.5 (#108)	54.5 (#23)	54.5 (#1)	136.3 (#1)	136.3 (#108)	27.5 (#1)	27.5 (#11)	27.5 (#108)	13.6 (#1)	13.6 (#11)	13.6 (#108)
Dose A. H. H., mgm.	65.0 (#120)	65.0 (#120)	65.0 (#120	65.0 (#120)	65.0 (#120)	65.0 (#107)	65.0 (#107)	65.0 (#107)	65.0 (#107)	65.0 (#107)	65.0 (#107)	65.0 (#107)	65.0 (#107)
Average weight of ovaries, mgm.	156.3	117.0	110.0	88.0	107.3	114.0	117.7	78.7	72.3	63.7	74.7	65.7	49.3
Activation	150%	95%	77%	29%	53%	78%	107%	78%	85%	95%	100%	91%	53%

hypophysectomized animal. Prolan has but a limited effect on the ovaries of the normal infantile rat. Hypophyseal hormone stimulates the infan-

TABLE 7

Effect on the immature rat ovary of prolan combined with hypophyseal preparations containing the gonad stimulating hormone but free of growth hormone

	DESIGNATION OF EXPERIMENT							
	377	373	372	375	389	388	369	399
Dose prolan, mgm...	13.6	0	13.6 (#337)	27.2	0	27.2 (#375)	54.5	54.5 (#369)
Dose A. H. H., mgm.	0	20.2	20.2 (#373)	0	65.0	65.0 (#389)	0	65.0 (#389)
Average weight of ovaries, mgm.....	32.3	25.7	32.7	32.7	13.7	43.3	52.0	43.7
Activation..........			None			Incon-sider-able		None

TABLE 8

Effect on the ovary of the immature rat of combinations of prolan with hypophyseal preparations containing growth but free from the gonad stimulating hormone

	DESIGNATION OF EXPERIMENT								
	375	220	446	450	486	485	499	272	501
Dose prolan, mgm.	27.2	0	27.2 (#375)	27.2 (#375)	54.5	0	54.5 (#486)	0	54.5 (#486)
Dose A. H. H., mgm............	0	112.5	45.0 (#220)	22.5 (#220)	0	24.0	24.0 (#485)	41.3	41.3 (#272)
Average weight of ovaries, mgm....	32.7	21.0	85.1	53.0	62.3	20.3	92.3	16.5	106*
Activation........			160%	62%			48%		70%

* These ovaries were composed of 0–3 corpora and 7–20 large cysts. In this experiment the activation can not be explained on the basis of the additive effect of so-called "follicle stimulating" and "luteinizing" hormones.

tile genital system of the hypophysectomized dog and rat to full development. Hypophyseal hormone is not limited in its action on the infantile ovary as is prolan. Prolan can be made to have a maximal effect on the

ovary by combining it with an hypophyseal extract, which by itself is not potent in accelerating sexual maturity.

We offer the following explanation of these facts. The gonad-stimulating hormone is present in the hypophysis in an inactive state. The active substance is formed from this "prohormone" by an activator. This activator is found in the most concentrated form as prolan in the urine of pregnant women. It may also be present in the hypophysis itself, even in the infantile hypophysis. But if the activator is present in the hypophysis it is normally separated from the prohormone somewhat as glycogen is separated from glycogenase in the liver. In the normal infantile animal its effect is limited by the small amount of prohormone present. If we add more prohormone to the infantile animal's own store, we can stimulate by prolan the development of ovaries that exceed normal physiological limits. Our purest growth hormone preparations are activated in this way. Hence the prohormone may be the growth hormone; we have at any rate not been able to separate it from the growth hormone. On the other hand growth-free gonad-stimulating hormone from the hypophysis can not be further activated by prolan, doubtless because it is already completely activated or maximally potent.

These explanations seem to us to best fit the experimental findings though we realize that there are other possible interpretations than those offered. The simplest explanation would seem merely that there is an additive effect when prolan is combined with hypophyseal hormone. We believe that our experimental work has excluded this possibility. The ovary weights stimulated by the combination are larger than what would be expected on the basis of an additive effect. Further, prolan can be combined with potent sex hormone preparation from the hypophysis without showing the activation effect. Most crucial of all, prolan activates hypophyseal growth extracts which by themselves show no gonad-stimulating effect at any concentration.[10]

We believe we have excluded also a simple physical explanation of the activation phenomenon (tables 2 A, B, C). The absorption, excretion, or possible destruction of prolan might be delayed by the combination, and therefore the actual amount of hormone available to the affected organ might be greater. If the hypophyseal component is replaced by a non-specific substance of similar physical make-up—e.g., liver—the activation reaction does not occur. Similar activation fails if the hypophyseal component is destroyed by chemical reaction or mild heat which does not sensibly change its physical character.

Furthermore we do not feel that we have grounds for assuming that our findings can be explained on the basis of different proportions of so-

[10] Even at five times the concentration used in the activation experiment.

called "follicle-stimulating" and "luteinizing" hormones in the two components used in the activation reaction.[11] For example it might be assumed that prolan contains more follicle-stimulating hormone and that the hypophyseal extracts furnish an excess of the luteinizing principle and that on combination of the two products a summation of these effects occurs. This assumes that the luteinizing hormone cannot act on an ovary unless it is first stimulated by the follicle-stimulating hormone. Although the large ovaries in the activation reaction are usually composed chiefly of corpora, this is not always true. We have had cases of activation in which the heaviest ovaries produced in a given experiment were composed almost entirely of large follicles and follicular cysts.

We believe that a recent observation of Hill and Parkes on ovulation in decerebrate rabbits can be explained on the same basis as our experiments. Hill and Parkes found that the injection of the urine of pregnant women (prolan) was able only in slight measure to replace the action of the animal's own hypophysis in provoking ovulation in animals decrebrated immediately after copulation. It was effective in only a small per cent of cases "4 inferior results" resulting from 19 trials. The ovulation was delayed longer than usual and less ova were freed. Even this minimal effect was not possible when placental extracts were injected into these decerebrate rabbits. On the other hand the placenta of pregnant rabbits, which had been injected previously with the urine of pregnant women, provoked ovulation in the decerebrate animals. We assume that the development of the gonads of the immature rat is a comparable phenomenon to ovulation in rabbits, and that hypophyseal hormone was necessary for ovulation in the rabbits used in the Hill and Parkes experiments; in other words, that prolan was unable to function except in combination with hypophyseal hormone and that the few positive cases of ovulation reported by them were due to minimal amounts of hypophyseal hormone still circulating in the blood of the decerebrate animals, which, combined with the prolan injected or stored in the placenta of the urine-injected animal, was adequate to provoke ovulation.

CONCLUSIONS

1. Prolan does not easily repair the gonadal deficiencies of hypophysectomized animals (dog, rat).
2. There is always a definite limit to the weight of the ovary which can

[11] After the present communication was written, the paper by H. L. Fevold, F. L. Hisaw and S. L. Leonard on the gonad-stimulating and the luteinizing hormones of the hypophysis (This Journal, 1931, xcvii, 291) appeared, but too late for discussion here. In it activation effects are described as the result of combining two hypophyseal preparations which gave respectively the follicle-stimulating and luteinizing effects. We intend to discuss this matter fully in a later paper.

be stimulated by prolan in the immature rat within a definite time interval (100 hr.).

3. Hypophyseal hormones completely repair the gonadal deficiencies of hypophysectomized animals (dog, rat).

4. The hypophyseal gonad-stimulating hormone does not show the limited effect on the ovary of the immature rat found to be characteristic of prolan. The development of the ovary provoked by the hypophyseal hormone corresponds to the dose level.

5. Only very small amounts of prolan, measured in terms of dry weight, are required to give the minimal effect on the ovary. The amount of hypophyseal hormone needed to give a minimal effect is always much greater. This is in contrast to the fact that higher doses of hypophyseal hormone provoke much greater ovary weights than can be obtained with any amount of prolan.

6. In our earlier experiments the effect of prolan was increased to the maximal effect obtainable by injecting hypophyseal sex hormone simply by combining prolan with small amounts of crude hypophyseal preparations, containing both gonad-stimulating and growth hormones. The combination of prolan was made with doses of hypophyseal preparations which, given alone, were minimal or just subminimal in gonad-stimulating effect.

Later we added prolan to hypophyseal extracts (growth hormone) which, when administered alone, were devoid of any effect on the ovaries of immature animals and in this way also secured maximal effects.

7. This activation effect is a specific reaction. If the hypophyseal extract is destroyed by heating, the combination of heated hypophyseal hormone with prolan is no longer any more effective than prolan alone.

8. Low concentrations of pure hypophyseal sex hormone combined with prolan do not result in activation effects. On the other hand, sex-free growth hormone is typically activated by prolan.

RELATIVE INEFFECTIVENESS OF PROLAN IN HYPOPHYSECTOMIZED ANIMALS[1]

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Smith has shown that at least four clear cut effects follow complete removal of the anterior hypophysis in the young animal (rat), namely, undergrowth, sexual infantilism, and impairment of the thyroid and adrenal cortex. He has shown that all the deleterious effects from hypophysectomy can be obviated by a substitution therapy[2] in the form of frequent implants of rat anterior hypophyseal substance. Reichert[3] has similarly been able to repair the growth and gonadal deficiencies of completely hypophysectomized dogs by implants of rabbit hypophysis. We now possess potent extracts of the anterior hypophysis which appear to selectively affect the growth and sex mechanisms respectively. Smith was able to show that our extracts rich in the growth hormone could restore growth in hypophysectomized rats without, however, affecting any of the other three deficiencies. Perhaps the most complete accomplishment in this respect was the growth which Reichert[4] provoked by administering our aqueous alkaline extract intraperitoneally to hypophysectomized puppies; these animals exceeded in growth their normal controls.

The discovery by Aschheim that the urine of pregnant women is capable of provoking precocious sexual maturity in young rodents, a response which hitherto had been provoked only by implants of anterior hypophyseal tissue, led us to hope that this substance, Prolan, might mature the

[1] Aided by grants from the Committee for Research in Problems of Sex of the National Research Council, and from the Rockefeller Foundation. These funds have been generously augmented by the Board of Research and the College of Agriculture of the University of California.

[2] Smith, P. E. The disabilities caused by hypophysectomy and their repair. Journ. Amer. Med. Assoc., 1927, lxxxviii, 158.

[3] Reichert, F. L. The result of replacement therapy in an hypophysectomized puppy: four months of treatment with daily pituitary heterotransplants. Endocrinol., 1928, xii, 451.

[4] Reichert, F. L. Effects of anterior pituitary extract upon an hypophysectomized puppy. Proc. Exper. Biol. and Med., 1929, xxvii, 204.

infantile sexual apparatus of hypophysectomized animals. Besides our desire to know whether Prolan could parallel the action of hypophyseal implants in the repair of gonadal deficiencies in such animals, we had an added interest in its injection, namely, to see whether repair of the thyroid and adrenal deficiencies paralleled gonadal repair.

A total hypophysectomy was done on three puppies when approximately six weeks of age following the method employed by Dandy and Reichert.[5] In all three cases within a few weeks it was evident from careful measurements of roentgenograms that growth stasis had resulted and that the operation was therefore complete. At various intervals after the operation, to be precise, at twelve, at two, and at six months respectively, high dosage Prolan administration was begun. In the case of the animal which had survived hypophysectomy for a year, daily high dosage for a month with Prolan produced no perceptible effects nor did a laparotomy at this juncture show that we had affected the infantile state of the ovary and uterus. The ovary had undergone an almost complete fibrosis and no follicles were discernible. Injections were resumed after the operation and continued for a period of 17 days. The dosage was higher than before but with similar non-effect. Autopsy at this juncture disclosed the other uterine horn as still threadlike and the ovary as small, hard, and white.

It seemed to us possible that the extensive regression and atrophy of the genital system might now have carried with it the lack of capacity to respond to a stimulus and that therefore such attempts should have been carried out within a short time after a complete hypophysectomy. For this reason a similar intensive Prolan dosage was given a puppy two months after a complete hypophysectomy, but in this younger case also no result was elicited.

In the meanwhile we had by simple procedures (re-solution and precipitation) purified and concentrated our Prolan so that it had approximately five times the strength of the material first employed. A puppy which had survived complete hypophysectomy for approximately six months and was in good health, was treated with the stronger Prolan. Fifty-three daily injections of about 30 cc. each were made without demonstrable effect on the sexual apparatus. An autopsy showed that both ovaries and uterus were infantile.

Inasmuch as the effect of Prolan in hastening sexual maturity has been ascertained in most cases by the use of small rodents (mice or rats) there existed the possibility, of course, of a better possible response in these forms. Therefore, a rat $3\frac{3}{4}$ months old and weighing 171 grams was hypophysectomized (Pencharz). Two months afterwards, the operation being demonstrably complete, 8 units of Prolan were given subcutaneously

[5] Dandy, W. E. and F. L. Reichert. Johns Hopkins Hosp. Bull., 1925, xxxvii, 1.

daily for six days without effect. The animal was allowed to rest; then dosage was continued for ten days longer. At the end of this period there was still no change in the vaginal lips and the vaginal smear and the animal was sacrificed. Ovaries and uterus were infantile. The detail of all four experiments will be found in the tabular protocols.

The lack of response of the genital system of these hypophysectomized animals to prolonged and repeated administration of Prclan of known potency indicates at any rate the incapacity of such animals to respond to a treatment which has pronounced effects on the ovary of the infantile normal animal.[6]

PROTOCOL

DATE	PROCEDURE	REMARKS
Dog H-92, Female shepherd		
April 28, 1930	Total hypophysectomy with cautery at base of brain—Dr. Reichert	7 weeks old; weight 3.6 kilos (H-93, female litter mate control, 3.7 kilos)
April 28, 1930, to February 19, 1931	X-ray measurements of bones and body weight taken every two weeks	Gained 0.4 kilo, skull increased 0.6 cm., sum of tibia and femur increased 3.2 cm., never lost first denture (H-93 gained 12.7 kilos, skull increased 6.5 cm., femur and tibia increased 20.0 cm., second denture July 30, 1930)
December 9, 1930	Prolan injections begun	6 months post-operative 53 daily injections of approximately 30 cc. each (10 cc. subcutaneously, 20 cc. intraperitoneally) Potency of prolan, 1/8 cc. = 1 R. U.
February 20, 1931	Died	Pneumonia. Age 49.5 weeks No change in vulva suggestive of an effect on genital system. At autopsy, ovary and uterus proved to be infantile (H-93 came into heat Dec. 9, 1930)

[6] Since the above was written, a purification and concentration of Prolan has been effected by us; with the administration of 400 RU's daily to hypophysectomized rats, some instances of response of the genital system have been secured.

PROTOCOL—*Continued*

DATE	PROCEDURE	REMARKS
	Dog H-46, Female	
October 24, 1928	Total hypophysectomy —Dr. Reichert	Six weeks old
October 14, 1929	Prolan injections begun	One year post-operative→ Injections were made intraperitoneally daily. Total of 385 cc. given in course of 4 weeks = 14± cc. daily Prolan potency, 12 mgm. per cc. (1 mgm. = 1 R. U.)
November 11, 1929	Injections stopped	Because no external evidence of influence on genital tract appeared
	Laparotomy performed	One ovary and fragment of uterus removed. *Ovary,* small, hard and white. In section, *ovary* almost completely fibrous, no follicles discernible Weight of animal, 5 kilos
October 22, 1930	Prolan injections resumed and continued for 17 days	35–40 cc. prolan injected, intraperitoneally and subcutaneously, daily
November 8, 1930	Died	Death result of a fall Autopsy showed *uterus* thread-like; *ovary* small, hard, white. Typical of animals hypophysectomized for this period of time
	Dog H-80, Female	
October 21, 1929	Total hypophysectomy —Dr. Reichert	Six weeks old; weight 2.6 kilos. (H-79, female litter mate control, 23 kilos)
December 21, 1929	Prolan injections begun	Two months post-operative 15 cc. prolan (potency as for H-46) injected intraperitoneally, daily
January 17, 1930	Prolan injections	No external changes in genitalia; vulva infantile Weight of animal, 3 kilos (H-79, 9.3 kilos) Animal not sacrificed

PROTOCOL—*Concluded*

DATE	PROCEDURE	REMARKS
Rat W-30, Female		
November 4, 1930	Total hypophysectomy —Mr. Pencharz	Age, $3\frac{3}{4}$ months; Weight, 171 grams
January 15, 1931	Operation to obtain control tissues	Left ovary, part of left uterine horn, one adrenal, and one thyroid removed. *Ovary*, infantile
January 19, 1931	Prolan injections begun	1 cc. daily, subcutaneously for 6 days Prolan potency: 1/8 cc. = 1 R. U.
January 25, 1931	Prolan injections stopped	No change in vaginal lips or in vaginal smear during period of injection or subsequent to it
February 16, 1931	Prolan injections resumed	1 cc. daily, subcutaneously for 10 days
February 26, 1931	Injections stopped	No change in vaginal smear nor in lips of the vagina Weight during injection period, 160 grams
May 11, 1931	Died	Weight, 148 grams. Ovary, infantile

RESPONSE OF EXPLANTED CARDIAC MUSCLE TO THYROXINE

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It seems to be well established from the work of Lewis and McEachern (1931), Priestley, J. Markowitz, and Mann (1931), and Yater (1931) that perfused hearts of thyroxinized rabbits pulsate at a much faster rate than the hearts of non-thyroxinized rabbits. Mann, J. Markowitz and Priestley (1931) found that the heart of a puppy that had been transplanted by vascular anastomosis into the neck of a larger dog beat much faster after thyroxinization of the host. These experiments indicated that the tachycardia of patients with hyperthyroidism is not due to stimulation of the accelerator nerves of the heart, as had been supposed, but to a direct effect on the heart. Yater (1931) performed experiments with perfused hearts of rabbits which showed that the action of thyroxine is on all parts of the heart, auricles and ventricles, and that the increase in rate is not dependent on the presence of the sino-auricular node. It remained only to be determined whether thyroxine acts on the muscle fibers directly or through the mediation of nerve ganglia or nerve endings. The present report deals with experiments performed to procure this information.

Since the surgical excision of the intracardiac ganglia is impractical, and even if it were practical there would still remain the so-called intermediary receptive substance of Langley, probably in a sensitized form, the only recourse available was the use of fragments of embryonic heart excised before the appearance of functioning nerve elements and kept alive by the modern methods of tissue culture. The most convenient preparation of this type is the explanted heart of the chick embryo kept alive in a suitable culture medium. Such fragments pulsate for many days, during which time there is a vigorous proliferation of fibroblasts in the culture medium and some proliferation of muscle cells. Ultimately, the latter die and only fibroblasts remain alive; as a consequence, the pulsations gradually become feebler and stop. In the interim the pulsating fragment of cells of the heart muscle is available for various physiologic experiments.

It was reported in a previous paper (Markowitz, 1931) that such fragments removed from two-day-old embryos were usually devoid of functioning nerve tissue, as judged by the response to epinephrine and acetylcholine.

Since this observation is consistent with the anatomic data of Szantroch (1930) that ganglia do not make their appearance until after the third day in hearts of chick embryos, it may be considered a safe premise that if thyroxine exerts its usual accelerating effect on the pulsations of a fragment it has accomplished this by direction action on the muscle cells. We have found that thyroxine accelerates the pulsations of cultures of cardiac fragments removed even from two-day-old chick embryos. It was possible to show quite clearly that the rate of pulsation of such fragments increased markedly following incubation with thyroxine, when, at the end of the experiment, they were beating vigorously but were utterly irresponsive to epinephrine and acetylcholine. We submit these experiments, therefore, as evidence that thyroxine accelerates the heart rate of intact animals by a direct effect on muscle cells, without the intervention of any nerve elements, or the so-called intermediary receptive substance.

During the research it happened that thyroxine was also added to cultures removed from older chick embryos (up to nine days). These are known to contain ganglia, and the existence of functioning nerve elements was readily demonstrable by the typical effects of epinephrine and acetylcholine on the rate of pulsation. The addition of thyroxine quickly accelerated the rate of such explants; the addition of epinephrine caused further acceleration, whereas acetylcholine stopped the pulsations for at least thirty seconds. In general, however, such preparations were no more sensitive to thyroxine than were nerve-free explants.

Methods of experimentation. It is hardly necessary to state that scrupulous asepsis was maintained throughout. Cultures were prepared from the hearts of chick embryos after the eggs had been incubated forty-eight hours or longer. To excise the hearts of very young embryos it was found necessary to use a dissecting microscope and very sharp instruments. The hearts were removed and cut into fragments about 1 sq. mm. in size. These fragments were explanted into Carrel flasks, type D, containing 0.3 cc. of heparinized chick plasma and 1.0 cc. of embryo extract.[1] Fifteen minutes after the medium with the embedded fragments had clotted, 1.0 cc. of embryo extract was placed on the surface. The cultures were incubated at 39°C. for four to twenty-four hours, during which time the rates of pulsation had become stabilized and could be counted prior to the addition of thyroxine. Usually fragments of each heart were placed in at least two flasks and one flask was kept as a control. Most fragments, soon after explantation, showed regular vigorous contractions which continued for days with only slight change in rate. Although each fragment varied

[1] Embryo extract was prepared (somewhat after the method of King, 1930) by placing an eight or nine-day-old chick embryo in 9.0 cc. of Tyrode's solution and shaking rapidly until the embryo was broken up. It was then centrifuged for ten minutes and the opalescent supernatant fluid was used undiluted.

little in rate of pulsation there were great variations in the rates of different fragments. Various methods were used to get a physiologically active solution of thyroxine that would be of uniform strength, simple to prepare aseptically, and of a pH that would not upset the reaction of the tissue culture itself. The method finally adopted was to weigh out thyroxine accurately in a small pyrex tube and add sterile distilled water in the proportion of 1.0 cc. of water to 0.02 mgm. of thyroxine. The tube was corked tightly and sterilized at 15 pounds pressure for fifteen minutes. When needed, it was shaken thoroughly and 0.1 cc. of this suspension was added to the culture. This culture, be it repeated, had 1.0 cc. of fluid embryo extract in addition to the solid matrix. Calculating that all the thyroxine went in solution the final dilution was 1:50,000, admittedly a very high concentration.

To count the pulsations the cultures were removed from the incubator from time to time and placed on the stage of the microscope which was enclosed in a hot-box kept at 39°C. In a few minutes after stabilization of the temperature the pulsations became regular and counts were made. After the addition of thyroxine, counts were again made at various intervals, and when the rate had markedly increased, 0.1 cc. of weak solutions of epinephrine and of acetylcholine were added to the fluid bathing the pulsating fragments. After each addition of epinephrine or acetylcholine this fluid was pipetted off and replaced by the same amount of warm Tyrode's solution. This was left on for a moment, then removed, to be again replaced by warm Tyrode's solution. As a matter of interest it may be stated that some of these cultures were subjected to further incubation, in which case fresh embryo extract was added.

Results. A few typical experiments will best illustrate our results. Fragments from a two-day-old embryo heart were explanted in the usual manner and incubated for eighteen hours. At this time the rate of one fragment was 120 for each minute and the other fragment in a separate flask had a rate of 85. To the latter flask thyroxine was added. Six hours later the rates were 92 and 70, respectively. In twelve hours the rates were 100 and 185, respectively. When acetylcholine and later epinephrine were added to the fragment that had responded to thyroxine, the rate remained constant at 185. By the next day the thyroxinized culture was not pulsating. Irrigation with Tyrode's solution caused it to resume beating at a rate of 218, which six hours later had increased to 240. By the next day the pulsations had stopped and no attempt was made to revive them. At this time the control was beating regularly at 85 although prior to this it had remained constant at a rate of 100 for each minute.

In a similar experiment on a fragment of the heart of a two-day-old embryo the rate was 150. Six hours after thyroxinization the pulsations were slightly irregular both in rate and vigor, but after twelve hours the

rate was regular at 185 for each minute. In twenty-four hours it had increased to 226. At this time it did not respond to epinephrine or acetylcholine; six hours later it was quiescent. The control fragment remained pulsating at about 100 and responded to the addition of epinephrine by a temporary increase in rate to 171; it did not respond to acetylcholine.

A four-day-old fragment responded to thyroxine by an increase in rate from 171 to 240. The addition of epinephrine caused a further increase to 300. A similar fragment with a rate of 120 responded to thyroxinization by an increase to 160. When epinephrine was added the rate increased to 240. Acetycholine caused it to stop pulsating for one minute and then it regained the rate of 150.

Comment. These experiments bring to light the inherently probable observation that thyroxine accelerates the pulsation of cells of the heart muscle in a manner that cannot be attributed to any intervening nerve tissue. It is of interest to mention certain other effects of thyroxine on cultures of the cells of heart muscle apart from this effect on the rate of pulsation. Although the massive injection of thyroxine given to a dog is not followed by pronounced tachycardia for twenty-four hours, the addition of thyroxine to cultures of cells of pulsating muscle is often followed by a well-marked effect in twelve hours. In the experiment mentioned, the rate of a nerve-free culture increased from 85 to 185 a minute in twelve hours. Twenty-four hours after the addition of thyroxine it was quiescent until irrigated with a little Tyrode's solution to wash away the products of fatigue, following which it promptly began to beat at a rate of 218. Six hours later the rate was 240 in spite of the addition of acetycholine. By the next day the pulsations had stopped and no attempt was made to revive them. Sometimes it was possible to observe the culture fibrillating. The addition of thyroxine had evidently caused a progressive rise in the rate, resulting in fibrillation and finally in paralysis. It was frequently noted (these experiments were discarded) that following thyroxinization, periods of irregularity might interrupt the uniform rapid rate of the explant. Thus the culture might beat rapidly for five seconds and resume its former rate, or it might beat rapidly for a few seconds, then stop for as long as forty seconds and resume some type of pulsation with or without pauses at a slower or faster rate. Another observation that is probably significant is that cultures which had been subjected to these large amounts of thyroxine did not pulsate for as many days as did the controls. We are inclined to attribute this to the exhaustion of the muscle cells and the accumulation of fatigue products. These observations have, of course, a bearing on analogous states of the heart of patients with hyperthyroidism.

This is not the place to consider the physiologic basis for the clinical syndrome that has come to be recognized as hyperthyroidism, except to indicate that such salient features as exophthalmos, tremor, and tachy-

cardia have, for decades, been attributed to sympathetic over-stimulation. It may be taken as definitely proved that this conception is erroneous for tachycardia. The newer knowledge of the sympathetic innervation of skeletal muscle renders it improbable that the tremor is sympathetic in origin. Leaving other considerations of the possible mechanism aside, it is unlikely that the exophthalmos is of sympathetic origin, unless the site of stimulation is very specific. It is true that stimulating the cervical sympathetic nerves of animals may cause exophthalmos, but it also produces dilatation of the pupil and unilateral constriction of the blood vessels. It is illogical to attribute the exophthalmos to general sympathetic stimulation in the absence of these other accompaniments. If there were such a clinical syndrome as violent sympathetic over-stimulation its picture would be that of overdosage of epinephrine, a picture with which every clinician has become acquainted. Except for the tachycardia, this picture had nothing in common with the symptoms of hyperthyroidism.

SUMMARY

A study was made of the action of thyroxine on cultures of pulsating fragments of heart muscle removed from two-day-old chick embryos, before the appearance of nerve elements. Thyroxine exerted its typical effect on the frequency of pulsation and contractility of such fragments, bringing about a progressively greater increase in rate ending in some cases in fibrillation and paralysis. These results constitute evidence that in clinical hyperthyroidism the tachycardia is due to the action of thyroxine directly on the myocardium and not on intervening nerve elements.

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STUDIES ON THE PHYSIOLOGY OF THE LIVER

XXIII. The Rôle of the Liver in the Destruction or Inactivation of Nicotine

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It has been shown by Priestley, Markowitz and Mann (1930) that the ability of the liver to destroy strychnine is many times greater than that of an equal mass of skeletal muscle. The question naturally arises whether the liver possesses this marked ability to inactivate other highly toxic alkaloids. We shall report here the results of our investigation of the part played by hepatic tissue in the destruction or inactivation of nicotine. This alkaloid was chosen for the reason that it can be estimated biologically in small quantities. The method for doing this depends on the familiar property of nicotine to raise blood pressure. If 0.5 mgm. of nicotine is injected intravenously it will raise markedly the blood pressure of a dog weighing 5 kgm. as will the injection of 0.1 cc. of 1:1,000 epinephrine. As in the case of epinephrine the blood pressure soon falls to the original level, and when the injections are not made more often than every fifteen minutes a remarkably uniform response can be obtained by repeated injection of the same amount over a period of two hours.

In our investigations the dog and the frog (Rana pipiens) were used. If the dog was used the methods employed were those described by Priestley, Markowitz and Mann. In brief, the nicotine present in the blood was assayed in terms of its vasopressor action after passage through a heart-lung, a heart-lung-liver, a heart-lung-limb circuit and also on its action on the normal and dehepatized dog. Sufficient nicotine was dissolved in a small quantity of Ringer's solution and added to the venous reservoir of the heart-lung circuit to make a concentration of about 0.1 mgm. for each cubic centimeter of blood. Appropriate samples of blood were removed at intervals and injected into an etherized dog weighing 5 kgm.; 5 cc. samples were used as a routine.

The effect of nicotine was observed on normal, splenectomized, par-

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tially dehepatized and totally dehepatized frogs following injection into the dorsal lymph sac of 0.1 mgm. for each 10 grams of body weight. The time required for complete recovery from the prostration produced by the injection was used as the criterion of the detoxifying ability of the frogs of the respective series. The frogs were used four days after operation. This precaution was taken to eliminate the frogs that were destined to succumb as a result of operation; Mann and his co-workers have shown that if totally dehepatized frogs live for four days they usually live several days longer.

REPRESENTATIVE EXPERIMENTS. Two liters of blood were obtained from healthy donors and this was added to the venous reservoir of a heart-lung circuit. A dog weighing 10 kgm. was then etherized and its heart and lungs were connected in the usual manner with the heart-lung circuit. With maximal dispatch the liver was later placed in circuit in the manner described by Priestley and his co-workers. At this stage a dog weighing 5 kgm. was etherized and the blood pressure was taken through the carotid artery and recorded in the usual manner; 200 mgm. of nicotine were now added to the venous reservoir. The effect of the nicotine on the heart of the heart-lung circuit is interesting. After the addition of the nicotine it became markedly slow and irregular for five minutes. Then it was accelerated for about five minutes following which it behaved as it did before the introduction of nicotine. As a control the first sample of blood for assay was taken from the venous reservoir. Subsequent observations were made with samples of blood coming from the liver. Samples (5 cc.) were taken about fifteen minutes apart and the effect on the blood pressure recorded after intravenous injection into the dog that was used for the assay. The first sample gave the predictable result, namely, the effect produced by 0.5 mgm. of nicotine. Samples coming from the liver fifteen minutes after the addition of nicotine showed large diminution in the effect of nicotine. Successive samples showed less and less action. Seventy minutes after the introduction of nicotine the effect on blood pressure of a 5 cc. specimen of blood emerging from the liver was not appreciable. The injection at this point of 0.5 mgm. of nicotine into the etherized dog provoked the usual rise in blood pressure (fig. 1).

It is apparent that the liver possesses the ability to remove large quantities of nicotine from blood that has been perfused through it. Whether the nicotine is absorbed or destroyed was not determined. The fact that it disappeared completely within seventy minutes would suggest that it was destroyed by the liver.

It might be supposed that the nicotine undergoes the same alteration either in the heart or lungs or in the perfusing medium. To test this we performed an experiment on a heart-lung preparation without the liver

in circuit. The amount of nicotine in the blood did not show any significant change over a period of two hours, as was tested by the effect on the blood pressure of another dog.

To compare the ability of skeletal muscle to inactivate nicotine we prepared a heart-lung-limb circuit. The venous reservoir contained 1,200 cc. of blood. After ligating the aorta, a cannula was placed rapidly in the femoral artery and rapid amputation at the hip was performed. The limb was placed in the incubator and the femoral artery was connected with the heart-lung circuit proximal to the peripheral resistance. To the venous reservoir containing 1,200 cc. of blood was now added 120 mgm. of nicotine, otherwise the experiment was conducted as in the preceding case. As judged by the influence on blood pressure the rate of destruction of nicotine was definitely slower by the hind limb than by the liver.

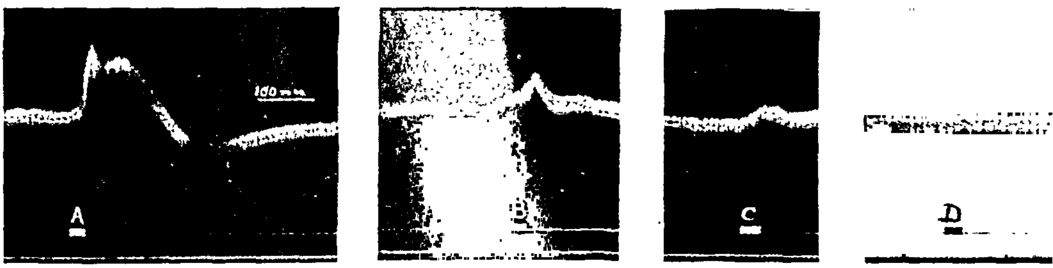

Fig. 1. The effect on the blood pressure of successive injections of 5 cc. of blood which contained approximately 0.1 mgm. of nicotine for each cubic centimeter at the beginning of the experiment. The response of the blood pressure to 5 cc. of blood removed from the venous reservoir previous to perfusion through the liver is shown at *A*. After the blood had been perfused through the liver for fifteen minutes the standard dose produced the response shown at *B*. The effect of the standard dose was weak when the perfusion had lasted forty-five minutes, *C*. At the end of seventy minutes there was no appreciable effect on the blood pressure, *D*.

The blood flow from the femoral vein was vigorous, amounting to about 150 cc. a minute. The blood issuing from the vein was venous in color. Fifteen minutes after the introduction of nicotine into the venous reservoir there was a sharp fall in the nicotine action to about half of its original value. Instead of diminishing progressively, however, it remained at this level in spite of the fact that the experiment was continued two hours. It would appear from these results that the nicotine diffuses and is perhaps absorbed by the muscular tissues, which may possibly account for the initial fall in the concentration. Much less nicotine disappeared subsequent to the initial fall than took place in the liver.

The effect of nicotine on the blood pressure of a dog was determined before and after hepatectomy. For this purpose a cannula was placed in the femoral artery. Following an injection of 1 mgm. of nicotine there

was a moderate pressor response (fig. 2). After the liver was removed the blood pressure was taken in the same manner. The response to a proportionate dose (0.95 mgm.) was much more pronounced than that before hepatectomy (fig. 3). The duration of the response before hepatectomy was about one minute, but after hepatectomy it was three minutes. This experiment also furnishes suggestive evidence that the liver plays a significant part in the inactivation of nicotine.

Further data were obtained by the study of normal, splenectomized, partially dehepatized and totally dehepatized frogs. The operative procedures were similar to those followed in previous investigations reported from this laboratory. It was found that an injection of 0.1 mgm. of nicotine for each 10 grams of body weight into the dorsal lymph sac of normal frogs caused complete prostration within one to two minutes.

Fig. 2

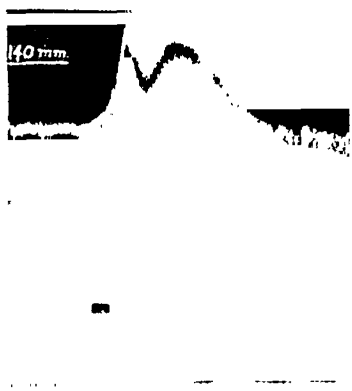

Fig. 3

Fig. 2. The blood pressure following an injection of 1 mgm. of nicotine into a dog weighing 12 kgm. previous to hepatectomy.

Fig. 3. The effect of 0.95 mgm. of nicotine after hepatectomy.

The initial response was a convulsive seizure of short duration, after which the frogs remained immobile until recovery or death. The time required for complete recovery, which was considered to have occurred when the frogs were able to hop after mild stimulation and right themselves when placed on the back, ranged from fifty-two minutes to three hours. The majority, however, recovered within two hours. The foregoing dose of nicotine was not lethal in a single instance when injected into thirty-six normal frogs.

The effect of the nicotine on totally dephepatized frogs was decidedly more toxic. In each instance the usual primary convulsion and prostration followed injection, but in many cases a second convulsion occurred from five to ten minutes after the first, following which the frog appeared dead, but usually the heart continued to pulsate slowly for varying

periods before stopping completely. Of fifteen totally dehepatized frogs ten died and five recovered. With one exception the time required for recovery far exceeded that for the normal, splenectomized and partially dehepatized frogs (fig. 4).

COMMENT. It is, of course, difficult to make the experiments on the heart-lung-limb the exact equivalent of those performed on the liver. However, the results obtained on a dog before and after hepatectomy were comparable in that the effect of the nicotine was more pronounced and of longer duration after removal of the liver. Furthermore, partially dehepatized and totally dehepatized frogs were more profoundly affected by a given dose of nicotine than the normal or splenectomized animal.

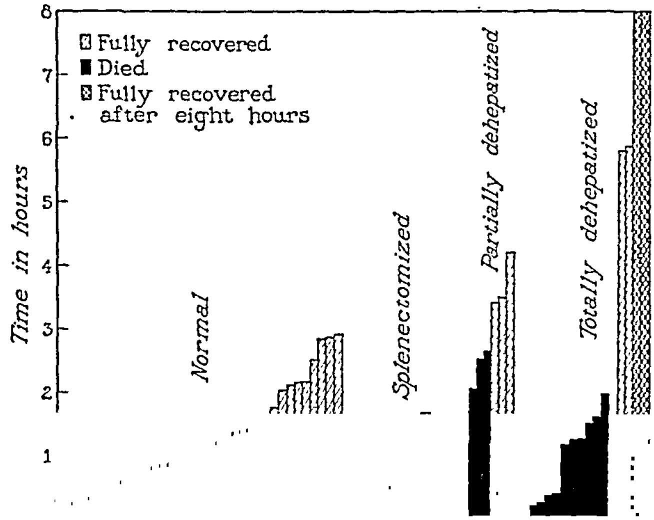

Fig. 4. The time of recovery or death of each of the normal, splenectomized, partially dehepatized and totally dehepatized frogs, following an injection into the dorsal lymph sac of 0.1 mgm. of nicotine for each 10 grams of body weight.

The fact that a high percentage of the totally dehepatized frogs succumbed, and that normal frogs survived following the same dose of nicotine indicates the importance of the liver in rendering this drug non-toxic. The long period required for complete recovery of dehepatized frogs indicates that the process of inactivating nicotine operates in the absence of the liver but proceeds at a much slower rate. From whatever angle the results are considered, the conclusion seems unavoidable that the liver is more active than other organs in removing nicotine from the circulation.

A number of suggestions may be offered as to the cause of this function of the liver to inactivate nicotine: 1, if the ability to destroy nicotine is a function of living tissue it is possible that an active tissue like the liver

would possess more of this ability; 2, since the liver structurally is like a sponge, the contact of the hepatic cells with the vascular circulation is an intimate one; the possibility for surface phenomena, that is, absorption, condensation, and dehydrogenation becomes facilitated, and 3, it is probable at any given moment that there is present in the liver a number of highly reactive chemicals which may easily combine with nicotine as it courses through this organ. Which of these methods is responsible for the marked ability of the liver to remove toxic substances from the circulation is a subject for further research.

SUMMARY

The ability of the perfused liver to destroy nicotine was studied by means of heart-lung preparations. The nicotine was estimated by its effect on the blood pressure of a small dog. Control experiments were performed by perfusing the hind limb in a similar fashion. It was found that the ability of the liver to destroy nicotine was definitely greater than that of an equivalent mass of hind limb. The action of a comparable dose of nicotine on the blood pressure of a dog after hepatectomy was much more pronounced than before hepatectomy. Following equal doses of nicotine, normal and splenectomized frogs recovered much more rapidly than partially and completely dehepatized frogs.

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DOES PHYSIOLOGICAL EXCITATION INFLUENCE PERMEABILITY IN STRIATED MUSCLE?

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In his book on permeability (1929) the senior author gave a summary of the relationship between excitability and permeability. On the basis of the work of Gildemeister, Ebbecke, Embden and others he concluded that increase in permeability ordinarily accompanies the transition from the resting to the excited state. It was assumed that we were dealing here with a general law of physiology holding for animal cells as well as for plant cells.

Some recent investigations seem to show that this assumption is not universally true. A great number of investigations, especially those of Embden and his co-workers have been performed on excised muscles with direct application of the electrical current. Although the effects of electrical stimulation were quite reversible and even subminimal stimuli evoked an increase in the output of phosphoric acid, it seems doubtful that the results obtained under these conditions can be applied to physiological excitation since Winterstein has shown that the local effects of electrical stimulation on the nerve are distinctly different from those of the propagated impulse. These experiments make it probable that a sharp distinction is to be made between electrical stimulation and physiological excitation. Since a propagated impulse set up by an electrical stimulation of the nerve corresponds completely to the physiological stimulation of the muscle, it seems necessary to investigate the question as to the permeability changes in muscle during excitation by means of indirect stimulation. Observations of Ernst and Csúcs (1929) and Mond and Netter (1930) had under such conditions negative results. These authors were unable to detect an increase in ion intake of perfused muscle when the muscles had been stimulated indirectly, and they concluded from their experiments that excitation is not accompanied by increase in permeability.

The question arises whether the negative results of Ernst and Mond might not simply be due to their methods. It is rather probable that permeability changes occurring in muscles under the influence of the physicochemical environment lead to significant changes in the intake of the effective substances. That holds, for instance, for Gellhorn's

studies on direct and indirect SCN contracture (1931). Therefore the question was investigated as to the permeability effects of indirect stimulation of the muscle by means of its functional reaction. Since the previous work of Gellhorn (1928) on KCl contracture seems to indicate that its strength, other conditions being equal, depends on the permeability of the muscle to KCl, the influence of indirect stimulation upon that contracture was studied.

METHOD. The experiments were performed on nerve muscle preparations (m. gastrocnemius) of R. esculenta from October 1930 to March 1931. The preparation was set up in aerated Ringer solution and connected with a light isotonic lever, which gave a magnification of eighteen-fold. The nerve was in a moist chamber and rested on platinum electrodes. Either faradic currents obtained from an induction coil with one dry cell in the primary current or condenser discharges were used for stimulation.

TABLE 1

*Height of contracture**

EXPERIMENT VI B	EXPERIMENT X B
mm.	*mm.*
1. 14	1. 11
2. *23***	2. 12
3. 18	3. *21*
4. *21½*	4. 14
5. 17	
6. *20*	

* "Height of contracture" is the distance the recording pointer rose above the base line, and represents the shortening of the muscle magnified eighteen-fold.

** The values in italics indicate the height of contracture during stimulation, the others being the control without stimulation.

The muscle was immersed periodically in a mixture of isotonic KCl (0.85 per cent) with Ringer's solution. The proportions were usually of the order of three parts KCl solution to five parts Ringer's solution, being varied for different preparations to produce a contracture of convenient magnitude. The proportion used in any one experiment was, of course, always the same. After each immersion the muscle was carefully washed in Ringer and then returned to aerated Ringer, where it was allowed to recover for ten minutes before another immersion. For control the muscle was merely immersed in the contracture producing solution. It was then immersed in this solution while being stimulated by break shocks of the inductorium (the secondary coil was set so that the make shocks were ineffective), or by discharges of a condenser (Scheminsky's apparatus). The frequency of stimulation used was between 60 and 120 per minute—frequencies which, for the durations used, caused no fatigue

contracture. The KCl contractures were recorded on a slowly revolving drum and allowed to reach a maximum.

Not all experiments showed an increased effectiveness of the potassium ions during stimulation. It was frequently difficult to obtain small contractions of the muscle, and large excursions of the lever were liable to destroy the effect, because the inertia of the falling lever during relaxation tended to stretch the muscle and counteract the effect of the potassium, since the tension set up by KCl is rather low. When conditions could be made optimal, however, the muscle revealed an increased susceptibility to potassium ions during stimulation. This is shown in table 1.

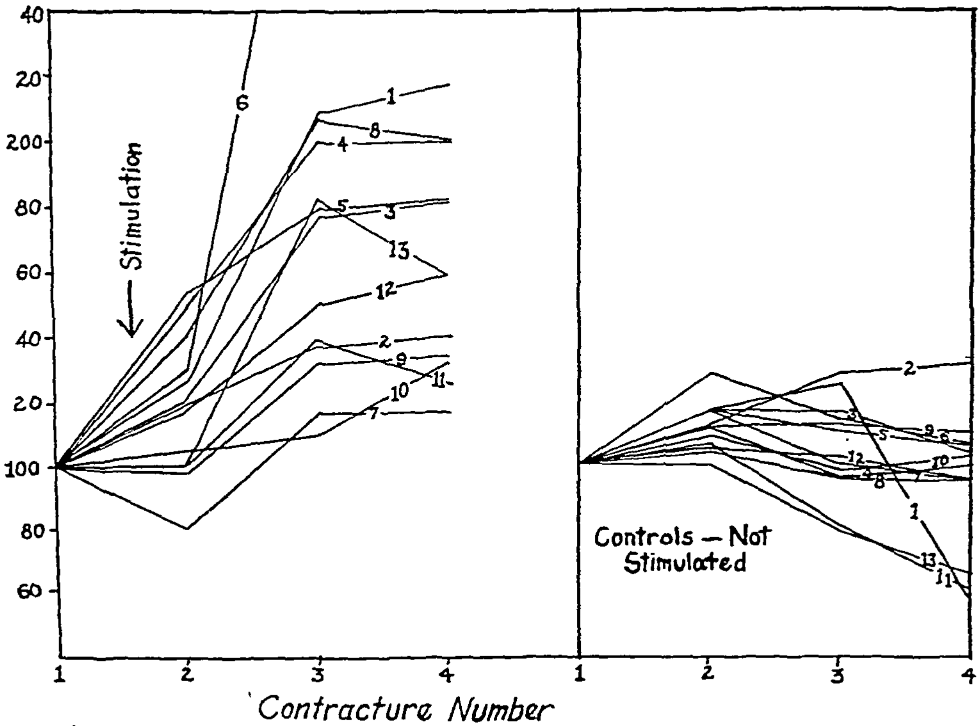

Fig. 1. The influence of indirect stimulation on the height of KCl contracture. Ordinate: Height of contracture expressed in percentages of the first one. Values (3) and (4) for curve 6 are 324 per cent and 354 per cent respectively.

In these experiments the two types of treatment of the muscle were alternated; i.e., first an immersion without stimulation, then with stimulation, then without, etc., the immersions without stimulation being for controls. The experiments show an increase in the height of the KCl contracture during stimulation.

A second type of experiment consisted in stimulating a nerve-muscle preparation *before* its immersion in the potassium solution and determining the influence on susceptibility to potassium ions. The procedure was as

follows: Two nerve-muscle preparations from one frog were used. First, each was immersed in the contracture producing solution and the contracture recorded. Then one muscle was stimulated with break shocks of the inductorium (which was set at a point where the make shocks were ineffective) at a frequency of 90 per minute. This stimulation was continued for five minutes, then stopped and a potassium contracture recorded, both in the stimulated and in the control muscle. It is notable that the threshold to electrical stimulation was not changed. Further potassium contractures were recorded in both muscles at intervals of twenty minutes thereafter. In very nearly all the preparations used, stimulation in this manner produced an increased susceptibility to potassium ions, as revealed by an increased height of contracture. In a few preparations this heightened susceptibility was not evident immediately after stimulation, but appeared twenty minutes later when a second contracture was recorded. Thirteen of these experiments were performed. The results are shown graphically in figure 1 in which the height of the first KCl contracture was taken as 100 per cent. The control value, obtained before stimulation, and subsequent contractures were expressed as percentages of the first. The graph shows very clearly the increase in susceptibility to KCl in the previously stimulated muscle.

The experiments show that indirect stimulation leads to an increased susceptibility of the muscle to KCl as is apparent in a study of KCl contracture. Since the muscle is permeable to K (compare Gellhorn, 1929), the conclusion seems justified that indirect stimulation of the muscle causes an increase in permeability. The experiments are in good agreement with observations of Thörner on degenerated muscles. In those, fibrillary twitches could be increased or provoked when the muscles perfused with KCl were stimulated indirectly.

SUMMARY

1. With two different experimental procedures it is shown that indirect stimulation of the muscle leads to an increased susceptibility to KCl contracture. This effect is obtained although the stimulation does not increase the threshold.

2. From these observations the conclusion is drawn that indirect stimulation leads to an increase in permeability of muscle.

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THE DIGESTION AND ABSORPTION OF RAW STARCH IN DOGS

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Several studies have been made concerning the digestion of raw starch. Langworthy and Deuel (1920) found the coefficient of digestibility in man to be nearly 100. More recently Roseboom and Patton (1928) found that dogs were able to digest raw corn starch in large amounts. Their method, similar to that of Langworthy and Deuel, consisted in the chemical examination of feces from dogs which had been fed large amounts of this carbohydrate. The feces were subjected to the iodine test for starch and were found to be uniformly negative. The material was then hydrolysed with dilute acid and tested for glucose. No glucose was found. They fed up to 75 grams raw cornstarch to dogs weighing about 15 kilograms, and concluded that dogs digested this quantity.

These authors also studied the amylolytic activity of extracts and secretions of dogs' salivary and pancreatic glands and believed that the pancreatic amylase was comparable in activity to that of omnivorous animals. Rosenthal (1929) fed raw starch to rabbits and other mammals and followed the curve of blood-sugar. He concluded that starch was digested and absorbed so slowly that the blood-sugar was not appreciably elevated. He fed important amounts of acid with the starch in order to delay its passage through the gut. More recently still Childrey, Alvarez and Mann (1930) have reported studies on digestion in dogs after colectomy. Their work suggested that there is little or no digestion of raw cornstarch in dogs and this interpretation has been made from their report. It is to be noted that these animals had a shortened digestive tube, that their findings were not based on chemical analyses, and that they were not definitely seeking for a proof of the digestibility of starch. They were searching for a "low residue" diet and were not chiefly interested in the chemical nature of the residue.

Because of this apparent disagreement we are led to report this experiment in which phlorizinized dogs were the subjects. We chose the phlorizin method as one which should definitely establish the facts of digestion and absorption of starch. This method on dogs has given good results in the hands of numerous investigators: Lusk (1912), Csonka (1916), and Olmstead (1920).

Two female dogs weighing 15.5 and 17 kilograms respectively were given daily injections of phlorizin in oil, 1.2 grams. They were also given several daily hypodermic injections of 0.2 cc. 1 to 1000 adrenalin hydrochloride. The urine was collected in 24 hour periods. The dogs were fed 250 grams of raw, ground, lean beef daily. The total nitrogen was determined by the Kjeldahl-Gunning method and the glucose by the method of Hawkins and Van Slyke (1929). The D/N ratios were calculated daily. When the D/N ratio approached 3.65, after a few preliminary days, the raw cornstarch was added to the ration for one day and determinations continued until the D/N ratio again approached 3.65. The results are indicated in the table. The data on the other dog were closely similar. The glucose derived from protein is obtained by using the factor N × 3.65. The remainder of the glucose or "extra" glucose is derived from the starch. The result shows a nearly quantitative digestion of the raw starch. Commercial cornstarch contains about 9 per cent of water.

Starch digestion, dog 1. Female, 15.5 kilos

Daily injection of 1.2 grams phloridzin in olive oil

DATE (1930)	FOOD	URINE						
		Amount in 24 hours	Nitrogen per cubic centimeter	Nitrogen total	Glucose from protein	Glucose "Extra"	Glucose total	D/N ratio
		cc.	*grams*	*grams*	*grams*	*grams*	*grams*	
April 16	240 grams lean meat							
April 17	240 grams lean meat	1,010	0.0134	13.53			78.70	5.81
April 18	240 grams lean meat	1,000	0.0155	15.50			65.00	4.19
April 19	240 grams lean meat	1,100	0.0144	15.84			58.60	3.7
April 20	240 grams lean meat + 50 grams raw starch	1,500	0.0094	14.10	51.47	38.53	90.00	6.38
April 21	240 grams lean meat	1,500	0.0087	13.05	47.63	7.12	54.75	4.19
April 22	240 grams lean meat	2,000	0.0081	16.20			59.00	3.64

SUMMARY

By the use of the phlorizin method it appears that dogs digest large quantities of raw cornstarch.

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THE EFFECT OF COMPLETE SUPRARENALECTOMY ON THE OESTRUAL CYCLE OF THE WHITE RAT WITH REFERENCE TO SUPRARENAL-PITUITARY RELATIONSHIP[1]

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The experimental study of suprarenalectomy in the white rat and its effect upon the oestrual cycle has interested many investigators in the last few years. Kitagawa, in 1927, stated that following double suprarenalectomy the oestrual cycle in young and adult white rats was lengthened, became irregular or was completely inhibited. Wyman reported similar results adding that the inhibition or lengthening of the cycle was dependent upon the degree of suprarenal insufficiency. No attempt was made by either author to study the factor or factors involved in the effect on the cycle. Contrary to these findings are those of Lewis, del Castillo and Schiffer and Nice who concluded from their experiments that the oestrual cycle remains undisturbed after both suprarenals are removed. These contradictory reports have led the author to re-investigate the problem in an attempt to determine the exact results of complete suprarenalectomy and the factors concerned.

I. Effects of complete suprarenalectomy upon the oestrual cycle of white rats. The vaginal contents of 121 adult female white rats (4 to 15 months old) obtained from three different colonies, were examined after the method of Long and Evans and only those with at least three normal successive cycles (4 to 6 days) were used for experimental work. Complete suprarenalectomy was performed in two stages with an interval of 3 to 4 days between the removal of the first and second gland. The operative technique was uniformly a dorso-lateral lumbar approach requiring 8 to 10 minutes from the beginning of ether anesthesia. The gland, its pedicle and all surrounding connective tissue and fat were removed and the peritoneal wall and the skin sewed up. All controls, 32 in number, were unilaterally adrenalectomized on the right or left side. In addition, a special control series of 11 female rats was subjected to the following treatment: splenectomy, unilateral suprarenalectomy, thyroidectomy, ovariectomy and salpingectomy, allowing some

[1] This problem was aided in part by grants from the National Research Council administered by Dr. F. L. Hisaw.

twenty-two days for the entire series of operations. This was done to determine more completely the effect of surgical trauma on the oestrual cycle and the success, in general, of the operator's technique. Daily body

TABLE 1

Effect of total suprarenalectomy on the oestrual cycle of adult white female rats

All were virgin females, 4–6 months old, except where noted.

EFFECTS ON OESTRUAL CYCLE AFTER REMOVAL OF 2ND SUPRARENAL	NUMBER OF RATS	OESTRUAL CYCLE		NUMBER DIED	PERIOD OF SURVIVAL	REMARKS
		Pre-operative	Post-operative			
		days	*days*		*days*	
1. Complete inhibition of oestrum	22	4.5–6 Av. 5.3	No cycles	22	8–17 Av. 11.1	5 were multiparae over 1 year old
2. One oestrual cycle followed by complete inhibition	38	3.5–5 Av. 4.4	6–12 Av. 7.7	36	9–19 Av. 16.6	12 were 8–12 months old. No cortical tissue found in two surviving rats
3. Two oestrual cycles followed by complete inhibition	14	4–5 Av. 4.7	7–11 Av. 8.0	13	14–26 Av. 19.3	10 virgin females 8–12 months old. No cortical tissue found in surviving rat
4. Several irregular or prolonged cycles	32	4–6 Av. 4.9	7–14 Av. 8.4	17	14–27 Av. 21.1	Accessory cortical tissue found in 8 of the 15 surviving rats
5. No effect	15	4–5 Av. 4.3	4–6 Av. 4.7	9	7–9 Av. 8.0	Accessory cortical tissue found in 3 rats
Totals and averages..........	121	4.7	8.0	97	15.2	

Death from total suprarenalectomy = 80.1%—oestrual disturbance = 87.6%

weights, vaginal smears and rectal temperatures were recorded of these 11 females for at least three weeks after the last operation. The remaining suprarenal was then removed from three of these rats. Daily body

weights were recorded also of most of the suprarenalectomized females used in this study.

Table 1 summarizes the results of all completely suprarenalectomized females with the exception of the few which died within 5 days after the removal of the second gland. Data on such rats were not included since the death during this period was regarded as an immediate post-operative effect. All recorded deaths were of rats showing the typical signs of suprarenal insufficiency in laboratory animals.

In 87.6 per cent of the cases, complete suprarenalectomy causes definite but varied effects upon the oestrual cycle. These have been grouped in order of severity in column 1, table 1. Almost all rats showing no more than two oestrual periods after the removal of the second gland died within a period ranging from 11.1 to 19.3 days. This is especially true of those females showing complete oestrual inhibition. Such rats also have the shortest longevity. In rats showing oestrual cycles, the period of survival is seemingly prolonged by the interval of a normal oestrum although the occurring cycle is much prolonged (average 8 days). Further, the vaginal smears of these females were usually atypical. When females showed several prolonged or irregular cycles, the period of longevity was longest and 43.8 per cent of these survived. Accessory cortical tissue was found in only 8 out of the 15 surviving rats of this group. These females survived and showed irregular cycles for at least six weeks.

In 12.4 per cent of the operated females, no appreciable effect on the oestrual cycle was noticed although strikingly 9 of this group died from glandular insufficiency. It is to be noted that the survival period of these females was exceedingly short. Of the surviving 6 rats, only 3 possessed accessory cortical tissue. Of all operated animals, 80.1 per cent died of suprarenal insufficiency, the period of survival ranging from 7 to 27 days (average 15.2 days).

Histological studies of the ovaries of females showing complete oestrual inhibition immediately following total suprarenalectomy, revealed solid masses of corpora lutea which apparently had persisted for a long time. No mature or large follicles were present, and only a few small undeveloped ova were seen. The germinal epithelium of the ovary was broken up and this and the region between the corpora lutea were often replaced by areas of degeneration. Macroscopically, these ovaries were about $\frac{1}{3}$ to $\frac{1}{2}$ the size of the control gonads.

The ovaries of females showing suprarenal effect 4 (see table 1) were, in most histological respects, similar to the controls. The microscopic size was approximately normal and cross-sections revealed only a few more larger corpora lutea. Mature follicles were just as numerous. In females showing effect 3, the gonads are apparently in an intermediate stage. The size varied slightly from $\frac{3}{4}$ to full size. The corpora lutea

were large and numerous and occasionally a mature follicle would be seen. The number of undeveloped ova was variable.

Table 2 shows the results of control female rats which have been unilaterally suprarenalectomized and 11 others which, in addition, had several more organs removed from time to time. The operations were performed at various stages of the oestrual cycle. It can readily be seen that simple operative procedures, even if performed in short successive intervals, do not disturb the oestrual sequence at all. The daily rectal temperatures remained normal at all times, the body weights fell slightly (3–9 grams) but rose again to normal on the second day after each operation. All control rats showed normal oestrual periods and increases in body weight for at least 25 days after the last operative procedure. The surgical trauma of these operations is thus a negligible factor in oestrual disturbance. After the removal of the second suprarenal gland in three of the 11 "special control rats," death from typical suprarenal insufficiency followed in 14, 15 and 22 days. The oestrual cycle was inhibited in all three animals for at least the last ten days of life.

TABLE 2

Effect of unilateral suprarenalectomy and other operative procedures on the oestrual cycle of adult control white rats

OPERATION PERFORMED	NUMBER USED	OESTRUAL CYCLE		NUMBER DIED
		Pre-operative	Post-operative	
		days	*days*	
Unilateral suprarenalectomy	32	3.5–6 Av. 5.2	4.5–6 Av. 5.3	0
Successive operations	11	4–5 Av. 4.8		
Unilateral suprarenalectomy			4–6 Av. 4.7	0
Splenectomy			5–6 Av. 5.5	0
Unilateral ovariectomy and salpingectomy			5–6 Av. 5.3	0
Unilateral thyroidectomy			5–6 Av. 5.6	0
Totals and averages..........	43	5.0	5.28	0

II. Experiments to determine the factors involved. In the second part of this research, an attempt was made to determine the factor or factors involved in the oestrual effect following total suprarenalectomy. In all the following experiments, the experimental rats consisted of only those females which definitely showed effects 1, 2 or 3 (see table 1) after both suprarenals were removed.

1. *Effect of transplants of adrenal cortex.* Nine experimental female rats showing complete oestrual inhibition for 9 to 12 days (at least two normal cycles) after complete suprarenalectomy were divided into groups of 3. Small pieces of suprarenal cortex varying in size from $\frac{1}{3}$ to $\frac{1}{2}$ were taken from the glands of normal rats and were transplanted into these females in the following locations: 1, intra-muscularly (dorso-lumbar and thigh musculature); 2, into the spleen, and 3, underneath the ovarian capsule. All members of the first two groups died of typical suprarenal insufficiency showing continued inhibition of oestrum. The 3 rats with intra-ovarial transplants survived, came back to normal weight and showed normal cycles (5–6 days) for at least 3 successive periods. Two of these females were bred and had normal litters of 7 and 8 respectively. By means of cortical transplants, normal ovarian function was restored. The ovary containing the cortical graft in these two females was removed three weeks after parturition. Both animals died 11 and 15 days later showing only one more cycle five days long.

2. *Effect of hypophyses of suprarenalectomized rats on immature ovaries.* The irregular occurrence of oestrual disturbance seen in table 1 suggested that the effect on the ovary is an indirect one. It was thought that possibly the hypophysis was the intermediary factor and that its gonad stimulating power in suprarenalectomized rats was decreased. The latter was tested by a method now commonly used, namely, implantation of the hypophysis into immature rats and weighing the hypertrophied ovaries.

A group of 36 suprarenalectomized male and female rats was used; the body weights of most of the animals and the vaginal smears of all females were recorded daily until definite signs of glandular insufficiency were seen. Rats in the last stages and others in approximately the midway period (animals with 9 per cent loss in body weight), were decapitated and bled to death. The hypophyses of two such animals of roughly the same body weight were then transplanted into the thigh musculature of an immature female rat (20–24 days old). The same procedure was followed with the pituitaries of 18 corresponding control rats. Five days after transplantation, the recipient females were killed and the two ovaries of each female were weighed together after the surrounding fat, connective tissue and oviducts were carefully removed. The following table summarizes the results of this experiment.

It is plainly seen in table 3 that total suprarenalectomy produces a definite and marked decrease in the gonad stimulating power of the pituitary gland. This effect is very marked in animals in the last stages of glandular insufficiency and is absent in suprarenalectomized animals that show a 9 per cent loss in body weight (roughly the mid-period of suprarenalectomy). The loss of gonad stimulating power of the anterior lobe of the hypophysis is greater in operated males and castrate males than in females. This is to be expected since the gonad stimulating content of the hypophyses of the former two is greater than in females.

TABLE 3

Effect of hypophyses of adrenalectomized adult rats on weight of immature ovaries

	NUMBER USED	NUMBER OF DAYS AFTER REMOVAL OF 2ND ADRENAL	AVERAGE WEIGHT OF IMMATURE OVARIES OF RECIPIENTS	PER CENT DECREASE IN GONAD STIMULATING POWER	REMARKS
			mgm.		
Control males	6		11.57		
Suprarenalectomized males	12	14–16 15.2	7.38	36.2	
Control castrate males	6		41.54		Males in castrate condition 4 weeks before experiment
Suprarenalectomized castrate males	10	12–15 14.3	26.67	35.7	
Control females	6		11.9		Only suprarenalectomized females showing effects 1 or 2 (see table 1) were used
Suprarenalectomized females	8	13–16 14.7	9.2	22.6	
Suprarenalectomized females in mid-period	6	10	12.1	No effect	

3. *Effect of injected hormones on the oestrual cycle of total suprarenalectomized female rats.* Following the results of the above experiment, it was natural to suppose that the effect of suprarenalectomy on the ovaries was by the way of the pituitary gland. If gonad stimulating hormone was supplied to female rats showing signs of suprarenal insufficiency and a complete inhibition of oestrum, would the ovary recover and oestrum occur? Would it recover following the injection of an ovarian hormone? These questions were answered by the results of 19 such experimental animals, 12 of which received injections of 0.5 cc. of Hisaw's gonad stimulating extract of powdered sheep pituitaries and 7 of which received

0.1 cc. or 1.5 rat units of theelin.[2] All injections were subcutaneous and were given twice daily for 3 to 5 days after the operated females showed complete oestrual inhibition for at least 12 days.

In every case, normal oestrum occurred as determined by the vaginal smear method. However, all 19 females showed fatal symptoms of glandular insufficiency and were exceedingly weak and unsteady 15 to 17 days after the removal of the 2nd suprarenal.

For control solutions, physiological saline and epinephrine chloride was injected subcutaneously into suprarenalectomized females showing similarly complete inhibition of oestrum for at least 12 days. The injections were carried out in essentially the same way —0.1 cc. of physiological saline and 0.5 cc. of epinephrine chloride (1:50,000) per injection given twice daily. Two groups of 6 rats each were used. All females died of suprarenal insufficiency with a continued oestrual inhibition.

4. *Cytological study of the hypophyses of adrenalectomized rats.* With some of the special stains and methods now available for studying the cytology of the anterior lobe of the hypophysis and depicting those cells presumably responsible for certain functions of this lobe, it was deemed advisable to save the hypophysis of some of the rats employed in each experiment. A detailed cytological study was made of this material by Doctor Bilstad and will soon be ready for publication. In short, it has been found that suprarenalectomy of adult males and females causes a decrease in the size and staining reaction of the oxyphil cells of the anterior lobe of the pituitary gland. The effect on the basophiles is not constant. Essentially the same change in the oxyphil cells occurs in the case of suprarenalectomized castrate males. It is concluded that complete suprarenalectomy produces definite cytological changes of the anterior lobe of the hypophysis.

5. *Effect of experimental loss of weight on the oestrual cycle of rats.* One of the well-known and most marked symptoms of suprarenal insufficiency is the gradual loss of body weight. It was believed that this symptom might be a contributory factor in the disturbance of oestrum following suprarenalectomy in addition to the absence of the cortical hormone. Perhaps with this weight loss there was also a serious decrease of vitamin intake (A, B and E). To test this, 14 female rats (135–170 grams) that showed at least three normal cycles, were placed in individual metallic cages and fed the ordinary Steenbock mixed diet. In addition, there were added twice per week 875 mgm. of dried powdered yeast (Northwestern Yeast Co.), 11 drops of cod liver oil, and a small piece of fresh lettuce. This special diet was always given first in tall dishes before the Steenbock mixture to insure complete ingestion and thus prevent any avitaminosis. Daily body weights and vaginal smears were recorded on all rats.

[2] Obtained by Dr. F. L. Hisaw from Parke, Davis & Co.

Of this group, two females were unilaterally suprarenalectomized and kept as controls and in two others, both glands were removed. Following this, the amount of Steenbock mixture fed to the remaining 10 unoperated females was decreased daily and was so graded that the loss in body weight almost paralleled the daily loss of the two doubly suprarenalectomized rats. The latter two died of typical glandular insufficiency in 13 to 14 days, one showing a complete oestrual inhibition and the other effect 2 (see table 1). The feeding of vitamins A, B, D and E to these rats showed no favorable effect on the ovaries. The per cent loss of body weight of these rats was 16.8; that for the group of 10 unoperated females ranged from 16.0 to 21.1 per cent (average 18.7 per cent). The unilaterally suprarenalectomized females showed a gain of 23.2 per cent in body weight for the same period. This large gain was undoubtedly due to the enriched diet (cod liver oil).

The results of the 10 non-adrenalectomized females given "the vitamins and decreased Steenbock diet" were striking. In 9 rats, the oestrual cycle remained uniform and undisturbed; in the remaining one, it was prolonged only one day which was in the 3rd oestrum of the 15-day period observation. Thus, it may be concluded that the gradual loss in body weight following double suprarenalectomy takes no appreciable part in oestrual disturbance.

Six of these ten animals were killed and their hypophyses preserved for cytological study. The other four were literally starved to death and seven days later were killed and the hypophyses, ovaries, uteri and vaginae saved. Just before death, these four animals showed an average fall in body weight of 37.8 per cent and postponed oestrum.

Discussion. Up to the present time there has been no uniform agreement in regard to the effect of total suprarenalectomy on the life of the white rat. A good deal of criticism has centered about the skill of the operative technique and the presence of accessory cortical tissue. The first factor can be controlled to a great extent so that after some practice, the technique, so far as tissue trauma is concerned, becomes negligible. Exceedingly short survival periods such as those reported by the early workers or even more recent ones (Freed, Brownfield and Evans) seem to us to point to operative procedures and to immediate post-operative effects. On the other hand, prolonged periods of 4 to 8 weeks and complete survivals are probably due either to the presence of accessory cortical tissue, which presumably takes over the function of the suprarenal cortex, or to failure in the first place to remove all of the gland with its pedicle and surrounding connective tissue. The latter is an important point since it has been shown by Pencharz and Olmsted and Pencharz, Olmsted and Giragossintz and others that small fractions of the cortex will proliferate and supply enough cortical hormone for the animal's economy.

It has been the experience of this laboratory following complete suprarenalectomies performed on more than 300 white rats of both sexes that these animals do not survive suprarenalectomy. This result is in accordance with the work of Pencharz, Olmsted and Giragossintz, Kutz and others and is true for both old and young rats. In the males used in this work, more deaths occurred (about 75 per cent) than one would ordinarily expect in view of the reported presence of accessory cortical tissue between the epididymis and the testis. The survival of female rats reported in table 1 is in part explained by the presence of accessory tissue confirmed histologically. However, in a few cases, no trace of accessories was found although the entire dorsal peritoneal wall especially in the mid-line from the diaphragm to the pelvis was carefully searched. Undoubtedly, cortical tissue was present and functioning in the body. The range in survival period for adult females was 7 to 27 days, the average being 15.2 days.

That the oestrual cycle in females white rats is disturbed following complete suprarenalectomy seems unquestionable. However, the effect is inconsistent and variable, which confirms the work of Wyman and Kitagawa. The most pronounced and consistent disturbances were seen in old multiparae and virgin females (8–12 months old). Perhaps in older animals, where presumably a higher level (in quantity) of hormonal activity prevails, a disturbance of endocrine equilibrium results in a sacrifice of the gonadal supply in favor of the more vital bodily processes.

In view of the inconsistent and variable effect on the ovaries, it seemed that the disturbance might be produced by some intermediary factor, perhaps the anterior lobe of the hypophysis. The results of the second part of this investigation support this idea. The mechanism involved seems to be a primary injury to the pars anterior following complete suprarenalectomy, which, in turn, is reflected on the ovary. This interrelationship of the pituitary and the adrenal cortex has been expressed by many workers who either studied clinical cases (Kraus, Moehlig and others) or who merely mentioned the idea in the discussion of related work. Smith made the first effective attempt to establish this relationship when he reported the pronounced decrease in the size of the adrenal cortex following hypophysectomy in rats. However, so far as the writer is aware, no experimental evidence has been published to show the result of total suprarenalectomy on the hypophysis. Such evidence would explain the irregular effect on the ovary noted in the first part of this paper and possibly the effect on the testis reported by Freed, Brownfield and Evans.

The suprarenal-pituitary relation is supported by the results of the second part of this paper. Successful cortical grafts (the ovary apparently being the most suitable location) restore normal body weight and ovarian

function. Removal of the graft results in the usual symptoms of glandular insufficiency and also disturbance in ovarian activity. Thus, we can be reasonably certain that normal ovarian function is in some way related and dependent upon the presence of the cortical hormone. The irregular occurrence of this disturbance as well as the varied effect naturally suggests the influence of some intermediary factor. This has been found to be the anterior lobe of the hypophysis on the basis of the following results: 1, decrease in the gonad stimulating power of hypophyses of suprarenalectomized rats (see table 3); 2, the cellular damage of such hypophyses seen in cytological preparations, and 3, the return of oestrum in suprarenalectomized females by injected extracts of the gonad stimulating hormone of the anterior lobe. When theelin was injected, another hormone present in normal ovarian activity that was apparently missing in suprarenalectomized females, normal oestrum also occurred. The results of injecting gonad stimulating hormone and theelin into experimental females merely show that an ovary will recover from an inactivated condition when the specific missing hormone is supplied. The injected material, however, did not prolong or save the life of the adrenalectomized rats. Injections of saline and epinephrine, as one would expect, were ineffective on the ovary and on the life of the operated females.

The cytological studies of hypophyses of experimental females further point to a functional glandular interrelationship with the suprarenal. When the period of post-suprarenalectomy was greatest, the cytological changes were most marked. Hypophyses of operated animals in the midway period (the point at which there is about 9 per cent decrease in body weight) showed little or no change. Undoubtedly, time is one of the essential factors. In short survival periods (9–12 days), it is easily possible that severe symptoms might intervene and cause the death of the rat before any possible pituitary disturbance could take effect.

It might well be argued that the ovarian effect following total suprarenalectomy is not at all specific and that factors such as temperature, loss of body weight, avitaminosis and surgical trauma might be responsible. This, of course, can be true when carried to extreme. However, all animals were given the same food, kept in the same more or less constant temperature room during the various seasons of the year and were operated on at different stages of oestrum with no appreciable effect on the oestrual cycle. Operative trauma is likewise of no effect as is seen in table 2. Experiments performed to determine the influence of possible avitaminosis and gradual loss of body weight (not exceeding 21 per cent) in unoperated females paralleling that of suprarenalectomized rats showed that both factors were negligible. The oestrual cycle strikingly remained uniform and undisturbed. Since the oestrual cycle was normal, it was concluded unnecessary to repeat the transplant experiment with

the pituitary gland of such experimentally starved rats. There is no reason to expect any significant decrease in the gonad stimulating power. From these feeding experiments, it is to be emphasized again that oestrual disturbance in suprarenalectomized rats is not due to avitaminosis since all the vitamins now regarded as necessary for normal oestrum were fed to operated females with negative results. The suprarenalectomized rats died and showed no return of oestrum.

SUMMARY AND CONCLUSIONS

1. Complete suprarenalectomy in the female white rat results in a suppression or irregular appearance of the oestrual cycle. This confirms the work of previous investigators. The effect, however, is inconsistent and occurs in only 87.6 per cent of the females.
2. It is also confirmed that the rat is no exception to the fatal symptoms of suprarenal insufficiency. In females, 80.1 per cent die from double suprarenalectomy.
3. Intra-ovarial transplants of suprarenal cortex maintain normal health and body weight of the rat. Pencharz and Olmsted's results are thus confirmed. These transplants also restore normal ovarian activity.
4. Complete suprarenalectomy in adult females, normal and castrate males, changes the cytological picture of the hypophysis of these rats and also produces a marked decrease in the gonad stimulating hormone of the anterior lobe.
5. Subcutaneous injections of theelin and of extracts of gonad stimulating hormone of the anterior hypophysis were effective in restoring oestrum although the females died from suprarenal insufficiency. Similar injections of normal saline and epinephrine chloride gave negative results.
6. The ovarian disturbance following complete suprarenalectomy is not due to operative trauma, avitaminosis (A, B, D, E) or to the gradual loss in body weight not exceeding 21 per cent.
7. In view of the inconsistent and varied effect on the ovary following double suprarenalectomy and the functional and cytological changes of the anterior lobe always coincident with marked ovarian disturbance, it is believed that an intermediate factor is present. This has been found to be the hypophysis and serves to explain the mechanism involved in the oestrual disturbance.

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PERIODIC VENTILATION AS INDUCED BY EXPOSURE TO HIGH PRESSURES OF OXYGEN

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Breathing an atmosphere low in oxygen quite commonly gives rise to periodic ventilation. In contrast to this are the results of experiments herein reported which were primarily designed for the study of oxygen poisoning in animals exposed to high pressure of oxygen. These experiments seem to be of particular interest, not alone because of the extremely long duration of the periodic cycles, but also because of the marked changes in volume flow of blood, blood pressure, pulse rate and blood color associated with the periodic fluctuations in ventilation.

The apparatus used was that described (Bean, 1930). The pressure chamber was supplied with an enclosed spirometer having accessory electrical apparatus which made possible the recording of respiratory ventilation on smoked paper. An especially constructed manometer was employed for the recording of the grosser changes in blood pressure. Due to its large column of mercury, the inertia was too great for the registration of the faster pulse rates. A small supplementary manometer enclosed within the chamber and provided with an electrical contact device in circuit with a recording signal magnet proved to be satisfactory. Volume flow of blood was recorded by the thermopile method as devised by Gesell and Bronk (1926). The glass thermopile vessel located outside of the pressure chamber made the observation of changes in blood color very convenient.

The oxygen used for these experiments was supplied from commercial pressure tanks. The chamber and spirometer were thoroughly washed out with fresh oxygen before each exposure. The pressures to which the experimental animals (dogs of about 10 kgm. weight) were exposed varied somewhat in the different experiments, but in most instances was about five atmospheres. This pressure, attained within a period of about four minutes, was maintained for varying lengths of time depending upon the reaction of the animal. Decompression was carried out in stages so as to eliminate the possibility of effects of effervescence which might arise from rapid decompression.

Prolonged exposure to high pressures of pure oxygen seems to have

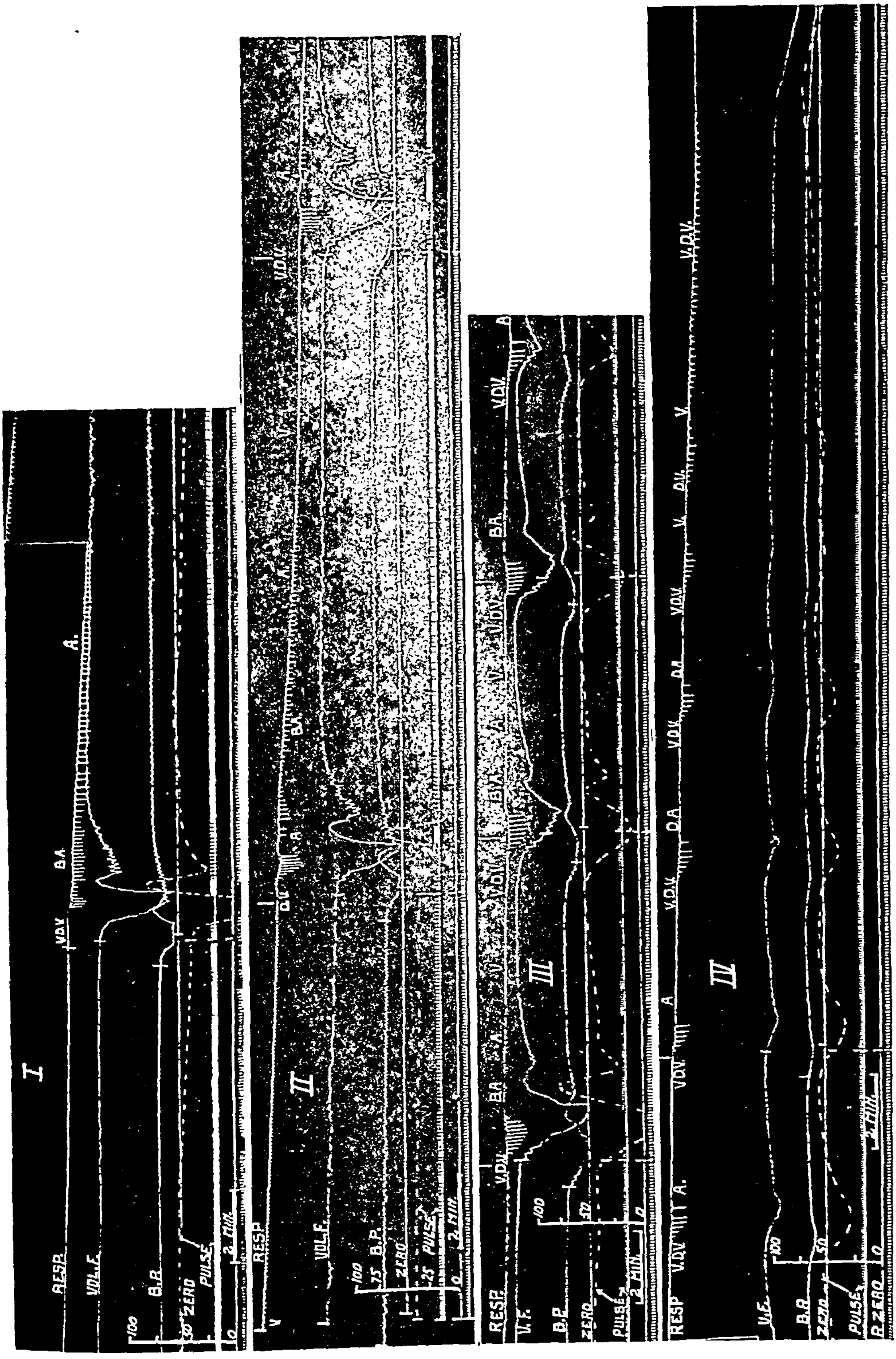

Fig. 1

been a contributing factor in the appearance of this periodicity yet some of the longer exposures failed to initiate any of these cyclic changes. In some instances, periodicity began during the time at which the oxygen pressure was highest and disappeared on decompression. In others, it appeared during the early stages of decompression and continued some time after atmospheric pressure of pure oxygen had been reached. Occasionally periodicity began only after decompression to atmospheric pressure had been completed. Periodic breathing was also observed at 3 and 4 atmospheres of pure oxygen in unanesthetized rats by Gesell (1923) in his studies on high oxygen pressure (personal communication).

Figure 1 shows records obtained towards the end of an experiment in which the animal was exposed to 3700 mm. oxygen pressure for sixty five minutes, followed by decompression (over a period of ninety minutes) to atmospheric pressure of pure oxygen. During this decompression the respiratory minute volume decreased continuously from 600 cc. per minute per kgm. body weight at the height of exposure to complete apnea on reaching atmospheric pressure. This apneic period—a part of which is shown at the beginning of record I of figure 1—continued for fourteen minutes. During this interval the color of the arterial blood (carotid artery) flowing through the thermopile vessel turned to a very dark venous hue, indicated on the record as V.D.V. The arterial volume flow of blood remained at a relatively constant value and the blood pressure likewise showed no significant change from its low level of 40 mm. Hg until toward the end of this apneic period. The pulse rate (the broken line obtained by plotting the rate as recorded in the third line from the bottom) maintained a constant level for thirteen minutes then suddenly dropped to zero. There was a complete absence of pulse for forty-five seconds as indicated by the pulse record and direct inspection of the mercury meniscus. At the end of this time, the heart rate rose rapidly to almost twice that obtaining before the initial drop. The accompanying changes in blood pressure and volume flow of blood were equally abrupt, similar, and almost simultaneous. The blood color, too, changed rapidly as indicated on the record. The long apnea preceding these alterations was broken by deep ventilation which began when the volume flow of blood, blood pressure and heart rate were at a minimum and the blood extremely venous in color. The depth of this initial ventilation decreased with the sudden rise of the pulse, volume flow and blood pressure, and a return of the blood color to a bright artierial (B.A. in record). A secondary augmentation in ventilation occurred with the second decrease in blood pressure, heart rate and volume flow, only to diminish again as the volume flow, heart rate and blood pressure returned to steady high levels. A tertiary increase in depth of ventilation which also gradually diminished is to be noted from the record. The primary and secondary groups concur with decreases in blood pressure

and volume flow of blood and heart rate; the tertiary with a sustained increase in blood pressure and volume flow of blood. The color of the blood became less bright and gradually a dark venous color following the tertiary increase in respiration. The subsequent changes, shown in record II of figure 1 (the continuation of record I), are almost a duplication of those of record I. Here too, there are to be seen a primary, secondary and tertiary increase in respiratory minute volume. The tertiary augmentation decreased in this case to an apnea. The color changes also were essentially the same as those seen in the first cycle. About fifteen minutes after the beginning of this second cycle of changes a third series occurred, but from this point on (records III and IV) the intervals between the successive cycles of periodicity became shorter and the changes less abrupt. The tertiary increase in ventilation diminished and disappeared in successive cycles and then the secondary disappeared. The primary increase became more gradual in its onset and decreased in depth associated with less abrupt changes in blood pressure, volume flow, and heart rate. Toward the end of record III and throughout IV none of the cycles showed a complete absence of pulse. The blood color changes became less sharp with a tendency toward a continued venous color. The end of record IV indicates the death of the animal.

Further variations in periodicity are shown in records A, B, C, D, E, and F of figure 2, each occurring in a separate experiment. Record B was taken from an experiment in which the animal had been exposed to 3700 mm. oxygen for fifty-four minutes during which time there had been marked hyperpnea. The pressure was lowered in three stages over a period of thirty minutes to 2068 mm. pressure of oxygen—the pressure at which the record was obtained. An apneic period of twenty minutes preceded the first ventilation shown in record B. This first period of increased ventilation occurred during a decreased volume flow of blood and when the heart rate and blood pressure were, so far as could be determined, zero; a second, and more prolonged period of ventilation was accompanied by an increased flow of blood, heart rate and blood pressure. The picture here resembles very closely some of the cycles shown in figure 1. In this experiment, as in figure 1, there was a very low general blood pressure—20 mm. was the highest value. The respiratory groups correspond to those in figure 1, spoken of as the primary and tertiary increases in respiratory ventilation. The primary occurred during a decreased volume flow and blood pressure, the tertiary during an increased flow and blood pressure.

In record D, figure 2, periodic changes came on when the oxygen pressure had been lowered from 3600 mm. to 1760 mm. Hg. The apnea in this case occurred during the periods of increased heart rate and blood pressure. Volume flow was not followed. The general level of blood pressure was

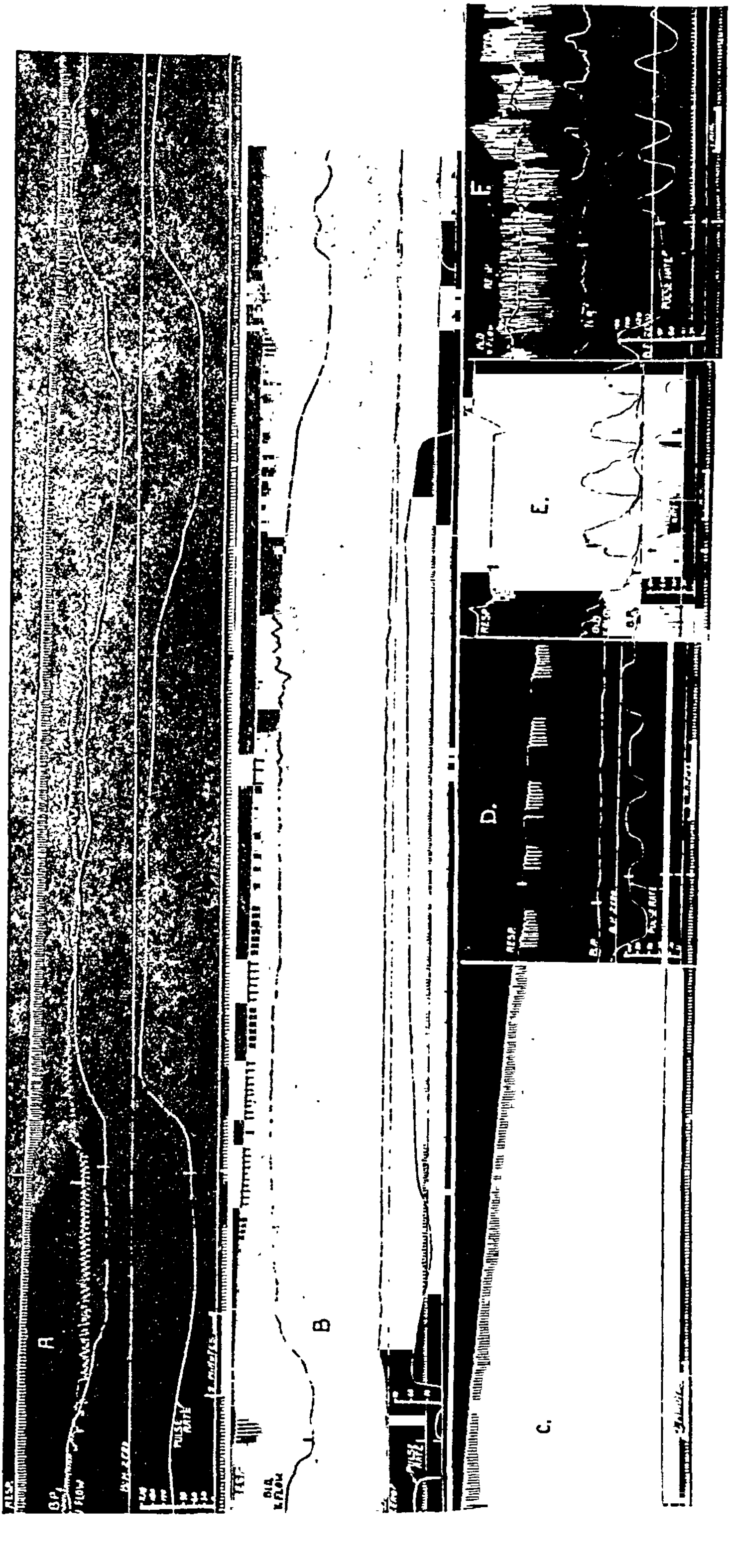

Fig. 2

only 36 mm. Hg. This periodicity corresponds essentially with that seen in record IV of figure 1 and is of a relatively simple variety.

Record E, figure 2, was obtained from an experiment in which the animal was exposed for thirty-five minutes to a pressure of 3680 mm. pure oxygen and after sixty minutes of decompression in three stages to 1000 mm. Hg. Apnea had come on at this pressure and had continued several minutes. The fourth stage of decompression was begun, but was only carried to 950 mm. Hg—indicated by the rise in the spirometer line at the beginning of the record (E, fig. 2). The blood pressure, volume flow and heart rate were decreasing when this reduction was made. The decrease in heart rate continued to a complete cessation as far as could be determined. Respiratory movements appeared during the period of cessation of pulse and decreased blood flow and blood pressure. The sharp rise in pulse rate and volume flow was accompanied by an apnea which continued to death ten minutes later. It is interesting that while respiration showed no further periodicity, marked periodic changes in pulse, blood pressure and blood volume flow continued to death. The heart, periodically breaking through the inhibition, apparently supplied enough blood to the cardiac center to maintain its function. The respiratory control mechanism, however, had apparently fallen to such a low level as to be incapable of further response. It should be noted that periodicity in blood pressure, heart rate and volume flow of blood preceded ventilation. This is another instance in which periodicity occurred during a very low blood pressure—the highest in the record was 24 mm. Hg and here, too, apnea was broken by ventilation which began during a period of decreased heart rate, blood pressure and volume flow of blood.

Record A, figure 2, was taken from an experiment in which the periodic cycles in ventilation, volume flow of blood and blood pressure occurred during exposure to 3700 mm. pressure of oxygen. These periodic changes (record A, fig. 2) were of about eight minutes' duration. No apnea occurred, but rather a relative decrease in ventilatory depth and rate during which time the pulse rate, volume flow of blood and blood pressure were lowest. This periodicity appeared after thirty minutes' exposure to the increased pressure and lasted until the first stage of decompression (about two hours later) at which point it became less distinct, but did not disappear entirely. It is of interest to note that during this periodicity shown in record A the blood pressure (120 mm. Hg) was considerably higher than that in the preceding experiment. Also the blood remained uniformly bright arterial. With later decompression and after breathing oxygen at 2300 mm. for forty minutes apnea came on and lasted twenty minutes. The blood pressure began to fall slowly and for fear of possible loss of the animal the oxygen pressure was raised to 2700 mm. Two breaths were taken and volume flow increased slightly, apnea followed for eight minutes

and blood flow began to fall again. The oxygen pressure was then lowered to 1500 mm. Hg. At a low point in blood pressure and volume flow ventilation returned and again showed periodicity, but of a different type than that which occurred at 3700 mm. pressure. The sequence of the cycle here corresponded closely to that shown in record III of figure 1. This periodicity continued for forty minutes during which time the pressure was lowered in one stage to 960 mm. Death occurred at this pressure.

The effect on the respiration of another animal which had been exposed to 3700 mm. pure oxygen is shown in record C, figure 2. This periodicity began fourteen minutes after raising the pressure and continued for one and one-half hours and through five stages of decompression which took another hour and fifteen minutes. The portion shown in C was recorded at a pressure of 2600 mm. Hg—one of the stages in decompression. This periodicity shows no apneic periods and the waves are relatively long, resembling those of record A, figure 2.

Record F represents still another experiment in which an animal had been exposed to pure oxygen at 3800 mm. Hg pressure for twenty-four minutes. The periods of decreased respiratory minute volume occurred during increased pulse rate, blood pressure and blood volume flow. There were no complete apneic periods but the decrease in respiratory minute volume was attended by a very shallow, rapid, panting type of ventilation. The rise in the level of the respiratory record in F was due to decompression to 2500 mm. Further decompression caused a shortening of the periodic changes and later a complete disappearance. In this instance the blood pressure was relatively high (130 mm. Hg at the lowest point) throughout, but the ventilation was quite unlike that seen in the other experiments in which periodicity was associated with a high blood pressure.

Discussion. The periodicity in respiratory ventilation shown in these records might be classified for purposes of discussion as being of two types which simulate in some respects those observed under different experimental conditions by Eyster (1906). The first, and probably more common variety, is exemplified in record IV of figure 1. Here the general level of blood pressure was low and increased ventilation concurred with a decrease in blood pressure, heart rate, and volume flow of blood. Each successive respiratory increase failed to bring about any sustained improvement in the animal, as indicated by the blood pressure, and death soon followed. A similar response is shown in record D of figure 2 which is from another experiment. The second type of periodic respiration is illustrated in record A of figure 2 where the period of increased respiratory ventilation was concurrent with an increased pulse rate, blood pressure, and volume flow of blood. The general level of blood pressure in this case was high and there was no apnea. Were the discussion to be confined to these records the conclusion that the two types of periodic respiration were

related to different physiological states of the animal would be justified. Casual inspection of records I and II of figure 1 might seem to invalidate such a conclusion since in these records there is a combination of the two types of periodicity occurring with what, at first sight, appears to be a common low condition of the animal. Careful study of the records, however, reveals that there were periods of improvement—more or less sustained—during which periodic respiration of the second type prevailed.

During the long apneic period (record I, fig. 1) the carotid blood had become very dark venous. This may have given rise to a vagal stimulation and a weakening of the heart resulting in a sharp fall in blood pressure and volume flow of blood. This sudden reduction in blood pressure and volume flow of blood in all likelihood led to a sudden increase in the stimulation of a depressed respiratory neuro-mechanism and brought about the initial or primary respiratory ventilation. This ventilation apparently improved the sensitivity of the controlling mechanism—or shifted the control to another level since the secondary increase in respiration occurred with a smaller fall in blood pressure, volume flow and heart rate. This secondary respiratory increase must in turn have brought about still further improvement in the condition of the animal since the blood volume flow and blood pressure subsequently rose to values higher than obtained during any preceding point of the record. The changed color of the blood also indicated an improved condition. It was during this improvement (which was sustained for some minutes) that the tertiary respiratory increase—periodic breathing of the second type—occurred. For some reason this improved physiological state was not maintained indefinitely and the animal slipped back to its former lower level. The series of events shown in record II was similar to that of record I. This second attempt at a prolonged recuperation ended like the first and when record III was reached the periodic respiratory response became purely one of the first type and continued through record IV to the death of the animal. With this disappearance of the tertiary increase in respiration, the sustained increases in blood pressure and volume flow also disappeared and there was a gradual drop in values in each successive cycle.

A somewhat similar response of another animal is shown in record B figure 2. In this instance, the secondary respiratory increase was absent, but the combination of the two types of periodic respiration might be explained in a manner similar to that above.

Analysis of these records showing a combination of the two types of periodicity then supports rather than disproves the belief that each was associated with a different physiological state of the animal. Either the respiratory controlling mechanism was behaving differently under the different conditions or there are different control mechanisms for these various conditions.

One of the puzzling observations in many of these experiments was the striking color changes accompanying periodic changes in respiration, heart rate, blood pressure and volume flow of blood. In an animal breathing pure oxygen it would seem as though the arterial blood would remain uniformly arterial in color. The lungs at the beginning of the long apneic period of record I, figure 1, were presumably full of oxygen—certainly not enough nitrogen could have come from the animal and accumulated in the lungs and spirometer during the experiment to be of any significance. The spirometer had been adequately washed out and previous tests had shown it to be free from carbon dioxide. The blood, while maintaining a relatively constant flow, gradually darkened in color to the end of the apnea at which time it had reached an extreme degree of venosity. In all likelihood the animal was then in a state of asphyxia. In support of this view is the marked slowing of the pulse—even to cessation—an effect quite commonly seen in asphyxia. Any rise in blood pressure which would have occurred as a result of asphyxial vasomotor constriction was overshadowed by the cardiac reaction resulting from stimulation of the vagus center and perhaps a weakening of the heart itself. This venosity of the blood was most probably due to a depletion of the oxygen in the lungs during the long apneic period.

Using figures based on human metabolism the basal consumption of oxygen in a 65 kgm. man of 170 cm. height with an R.Q. of 0.8 is found to be 14.5 liters per hour or 3384 cc. in fourteen minutes (DuBois, 1927). With the lungs filled with pure oxygen there would be nearly complete depletion of the oxygen at the end of this period. If we assume that the same relation of lung capacity and metabolism is roughly approximated in the dog, the extreme venosity at the end of the fourteen minute apnea is explained.

Dautreband and Delcourt-Bernard (1928) suggest that a constriction of the capillaries of the brain was a contributing factor in the appearance of the Cheyne-Stokes respiration they observed during administration of pure oxygen at atmospheric pressure. In view of the changes in blood color and volume flow of blood it is not likely that such was a contributing factor in the periodicity seen in the present experiments.

The periodic changes which were observed in our experiments were without exception attended by alterations in heart rate, whether the periodicity in ventilation involved apnea or not. Greeley and Greeley (1930) found that periodic breathing without apnea may also occur in the absence of alteration in heart rate.

SUMMARY

Exposure of animals to high barometric pressure of pure oxygen may give rise to periodic breathing, periodicity in heart rate, blood pressure, volume flow of blood and blood color changes.

The periodicity observed was especially striking in that there occurred, quite frequently, very long apneic periods—twenty minutes in some cases, complete cessation (so far as was determinable) of pulse with accompanying fall of blood pressure to zero and abrupt decreases in volume flow of blood. The color of the carotid blood changed periodically from a bright arterial to a very dark venous hue.

It was found that this periodicity might appear at the height of exposure to the increased pressure and subside on decompression. It often appeared in some stage of decompression or after the pressure had been lowered to one atmosphere of pure oxygen.

The periodic ventilation was of two types, one in which the ventilation was increased during a fall in blood pressure, volume flow of blood, and slowed heart rate,—a second in which the augmentation in ventilation occurred during a period of increased volume flow of blood, heart rate and blood pressure.

It is suggested that the two types of periodicity were perhaps due to difference in activity of the respiratory controlling mechanism or activity of different parts of the same mechanism under different physiological states of the animal involving flow and blood pressure.

Computation indicates that the changes of color which occurred in the blood were primarily a result of a depletion of the store of oxygen in the lungs giving rise to asphyxia.

The broken coördination of the dual function of the hemoglobin due to high oxygen pressure or asphyxia may play a rôle in the production of periodicity as observed in these experiments.

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A COMPARISON OF THE RESPONSE OF THE ANESTHETIZED DOG TO LOWERED ALVEOLAR OXYGEN DURING UNIFORM ARTIFICIAL VENTILATION AND DURING NORMALLY CONTROLLED VENTILATION[1]

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In an earlier series of experiments (Gesell, Krueger, Gorham and Bernthal, 1930) various respiratory phenomena were studied under numerous conditions, such as lowered alveolar oxygen, intravenous injection of sodium cyanide, hemorrhage, intravenous injection of sodium bicarbonate, hydrochloric acid, methylene blue, etc. Among the factors studied were carbon dioxide and oxygen content of expired air, total elimination of carbon dioxide and total absorption of oxygen, expiratory quotient, lactic acid content of the blood, carbon dioxide capacity of the blood, hydrogen ion concentration of the blood, volume flow of blood, mean arterial blood pressure, oxidative energy, non-oxidative energy, the relation of shortage of oxygen consumption to accumulated lactic acid, the relation of excess oxygen consumption to the disappearance of lactic acid, the relation of total elimination of carbon dioxide to that required by oxidations, the relation of the lactic acid content of the body to the liberation and retention of carbon dioxide, the proportion of fixed acid combining with bicarbonate and the proportion reacting with other buffer systems. In all of these experiments pulmonary ventilation was maintained at a known uniform volume.

We have now made a similar study during administration of gaseous mixtures low in oxygen and during intravenous injection of sodium cyanide, but in the present experiments the chest was kept intact and pulmonary ventilation was under the control of the respiratory mechanisms of the animal. The animal was, therefore, capable of controlling to some extent the oxygen and carbon dioxide pressure of the alveoli and tissues. Obviously the results might differ in very essential details from those in which ventilation was uniform.

Data of three experiments on three different dogs under morphine urethane anesthesia are presented in figures 1, 2, and 3 and tables 1, 2, 3, and 4. In one experiment a 7.24 per cent oxygen mixture was administered for

[1] Preliminary report: Proc. Soc. Exper. Biology and Medicine, 1930, xxvii, 849.

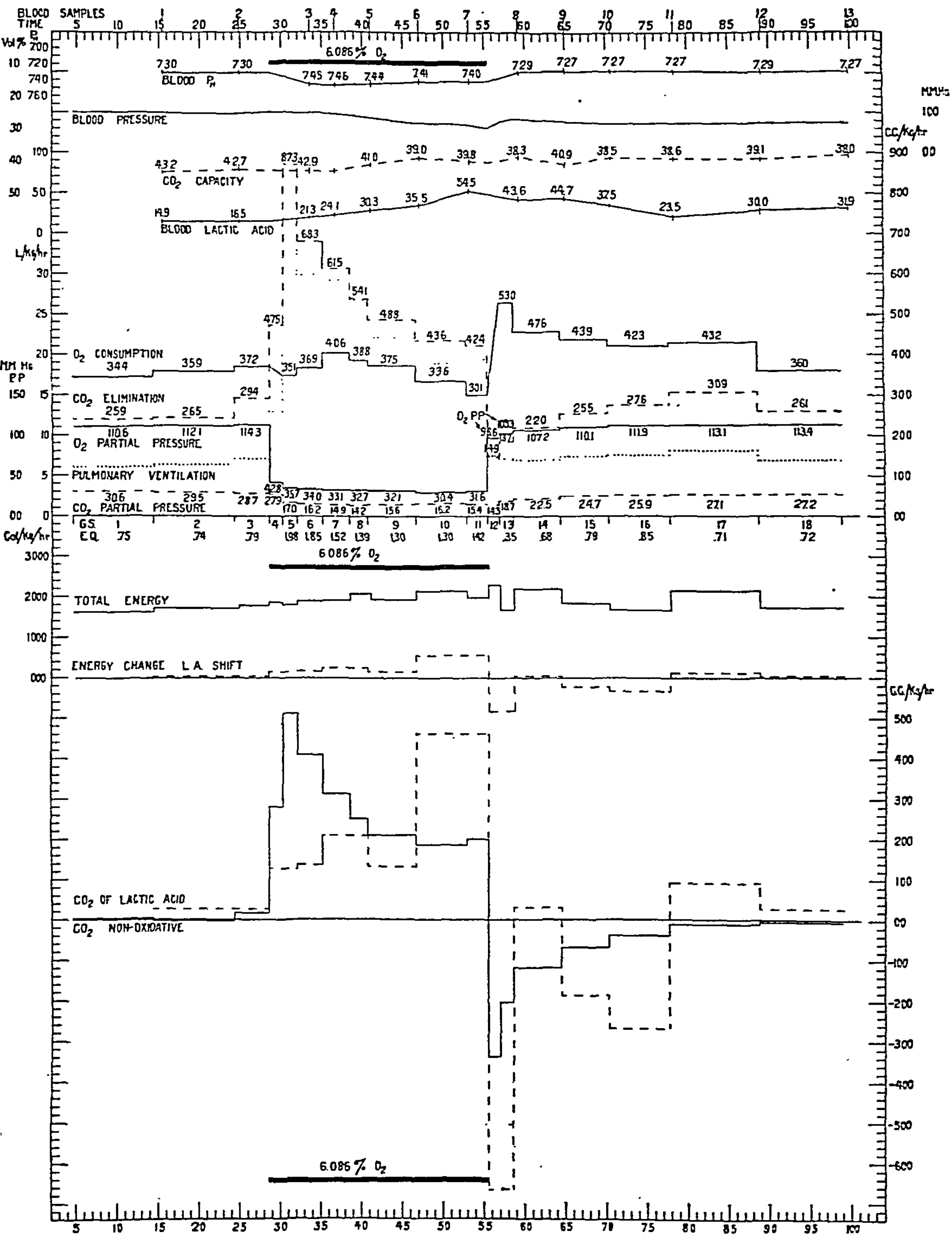

Fig. 1. Experiment 1, May 6, 1930

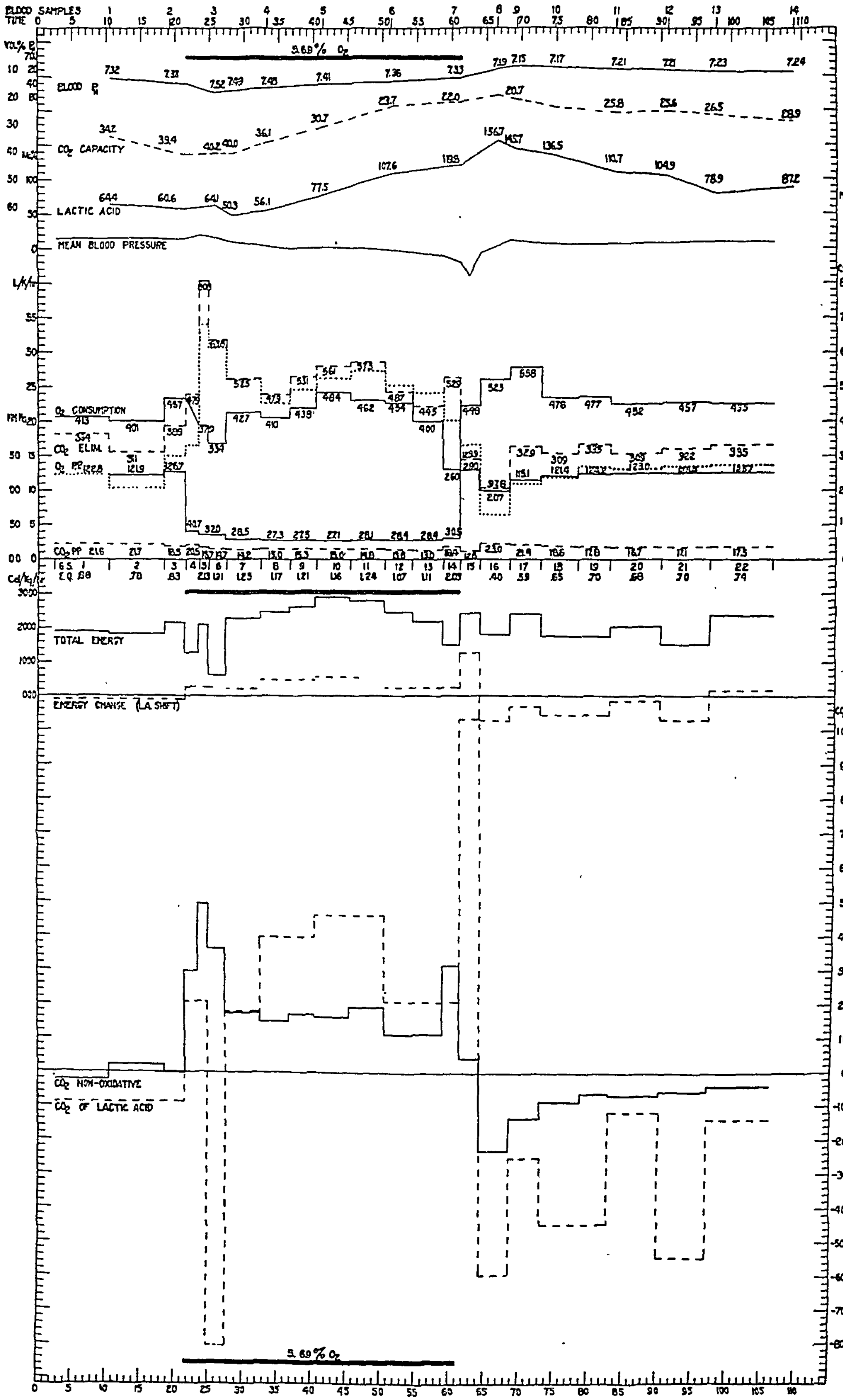

Fig. 2. Experiment 2, June 2, 1930

25 minutes and 8 seconds, in another 6.09 per cent oxygen mixture was administered for 26 minutes and 48 seconds and in the other a 5.69 per cent oxygen mixture was administered for 39 minutes and 50 seconds.

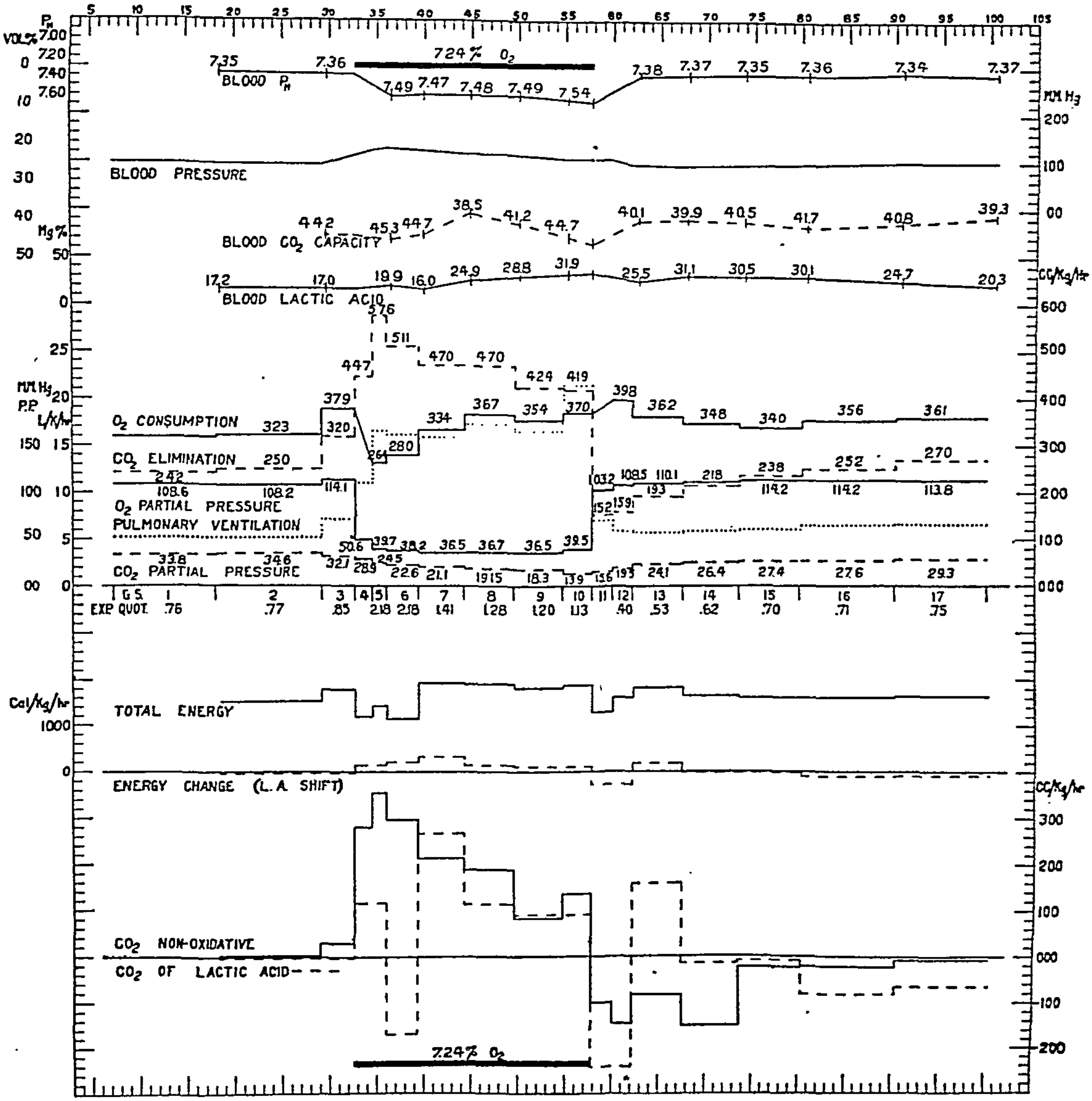

Fig. 3. Experiment 3, April 29, 1930

The total expired air was collected in rubber bags of suitable size connected by a three-way valve with the expiratory outlet of an inspiratory-expiratory valve. After allowing the collected samples to come to room temperature, two 50 cc. samples were withdrawn for analysis and the

volumes of the expired samples determined with a gas meter. For methods of computation and analysis the preceding papers should be consulted.

Experiment 1. Administration of the 6.09 per cent oxygen mixture led to a drop of expired oxygen pressure in the first two minutes from 112.1 mm. Hg in G. S. 2 to 35.7 mm. Hg in G. S. 5. The pressure then fell more slowly to a minimum level of 30.4 mm. in G. S. 10. During the initial period of lowered alveolar oxygen, pulmonary ventilation increased about five-fold, from 7,000 cc. per kgm. hour in G. S. 2 to about 36,500 cc. in G. S. 5. After the first three minutes of augmented ventilation the movement of air decreased again, but at the end of the period of lowered alveolar oxygen it was still approximately three times the preadministration ventilation. Oxygen consumption was not markedly affected—it first decreased, then increased above normal and finally decreased again below basal values. During this period of augmented ventilation the carbon dioxide pressure of expired air decreased from 29.5 to 14.2 mm. Hg. The curve of carbon dioxide elimination shows an increase from 265 cc. per kgm. hour while breathing room air, to 873 cc. during the initial period of augmented ventilation. Elimination then fell off along with pulmonary ventilation to 424 cc. per kgm. hour. The abrupt increase in carbon dioxide elimination occurring at the beginning of the period of lowered alveolar oxygen was associated with a corresponding abrupt increase in the expiratory quotient from 0.74 to 1.98. As ventilation diminished the expiratory quotient receded to somewhat lower levels (1.30, 1.30, and 1.42), but was still considerably higher than the preadministration values. The greater expiratory quotient, most probably, is primarily a resultant of three variables—increased pulmonary ventilation, augmented cardiac output and increased formation of lactic acid. Note the curve of carbon dioxide equivalent of lactic acid and compare this with the curve of non-oxidative carbon dioxide elimination.

It will be seen that lowered alveolar oxygen led to an immediate increase in the lactic acid content of the blood. This increase was maintained throughout the entire period despite the fact that oxygen consumption was temporarily increased. The increase in blood lactic acid may perhaps in part be attributed to the lowered hydrogen ion concentration of the blood (Macleod, 1921). On the other hand excessive oxygen consumption is by no means indicative of an adequate supply of oxygen. As a matter of fact the record indicates that the animal was deteriorating though oxidations were relatively high, for toward the close of the period of lowered alveolar oxygen, oxygen consumption again fell below the normal basal levels. Lactic acid accumulation was then augmented still more. Comparing the curves of carbon dioxide capacity and lactic acid content of the blood, it will be seen that the carbon dioxide capacity of the blood diminished at a rate slower than the accumulation of lactic acid, indicating as in the preced-

ing series of experiments that only part of the lactic acid reacts with bicarbonate base. Despite the accumulation of lactic acid and reduction in bicarbonate buffer base the blood turned more alkaline to the extent of 0.16 pH. The disproportionate blowing off of carbon dioxide and the lowered oxygenation of hemoglobin appear to be the main contributing factors to this effect. The further increase in blood lactic acid and the lowered ventilation must have contributed to the subsequent increase in hydrogen ion concentration from pH 7.46 to 7.40.

With readministration of room air there was a general reversal of conditions. During a period of two gas samples (13 and 14) pulmonary ventilation fell to the preadministration level and thereafter remained on the whole somewhat above the normal level. The increase in alveolar oxygen pressure was accompanied by an immediate overshooting of oxygen consumption, well sustained, but most pronounced in samples 12, 13, and 14. This period of greatest oxygen consumption was accompanied by the lowest oxygen pressure of the expired air (96.6, 103.3 and 107.2 mm. Hg) occurring during administration of room air and is accounted for by the subnormal ventilation and the supernormal absorption of oxygen. As pulmonary ventilation increased and oxygen consumption diminished the pressure of expired oxygen again equalled that of the preadministration period.

With readministration of room air the carbon dioxide pressure of the expired air fell from 15.4 to 14.3 mm. Hg and carbon dioxide elimination dropped from 424 cc. per kgm. hour to 149. From this point on, both the partial pressure of expired carbon dioxide and the total elimination of carbon dioxide increased to superbasal levels. The changing relations of oxygen consumption and carbon dioxide elimination which are easily followed in the graph led to rapid changes in the expiratory quotient. The most pronounced change occurred from gas sample 11 to gas sample 13 when the quotient dropped from 1.42 to 0.35. Except for gas samples 15 and 16 the expiratory quotient was lower than the normal preadministraion values. Since the lactic acid of the blood is seen to diminish on readministration of room air, the reduction in the expiratory quotient is conceivably due to the the retention of carbon dioxide by the base liberated in the disappearance of lactic acid as well as to the reduction of pulmonary ventilation and absorption of carbon dioxide by the desaturated tissues.

On readministration of room air the hydrogen ion concentration of the blood increased above normal despite the previous hyperelimination of carbon dioxide and despite the hypernormal ventilation. The persistently higher lactic acid values are of interest in this connection.

In experiment 2 a gaseous mixture with a lower oxygen content was administered and some of the effects were correspondingly greater. With the fall in oxygen pressure of the expired air from 121.9 mm. Hg at gas

sample 2 to 32.0 mm. at gas sample 5, pulmonary ventilation increased 226 per cent from 15.02 liters per kgm. hour to 34.12 liters. This increase will be seen to be less than in the preceding experiment despite the lower oxygen pressure of the administered gas. As in experiment 1, augmentation of ventilation was the greatest at the beginning of the period of lowered alveolar oxygen. This was followed by a decrease up through sample 8 and a second increase with the maximum during gas sample 11. The oxygen pressure of the expired air after reaching the low value of 27.3 mm. Hg at gas sample 8 remained at a relatively constant value. Oxygen consumption again showed an initial fall to 334 cc. per kgm. hour in gas sample 6 after which it increased to supernormal levels reaching a maximum of 484 cc. per kgm. hour in gas sample 11. From that point on to the end of administration, oxygen consumption rapidly fell to 260 cc. per kgm. hour. The fall in carbon dioxide pressure, beginning with lowered alveolar oxygen, was associated with an augmented elimination of carbon dioxide running roughly parallel with pulmonary ventilation. The expiratory quotient rose to the high level of 2.13 to fall again to 1.07 and 1.11 in gas samples 12 and 13. At the very end of the period of lowered alveolar oxygen the quotient rose again to 2.03. This final increase appears to be due to the sudden collapse of the animal associated with a reduction in oxygen absorption and an absolute increase in carbon dioxide elimination. The initial decrease in the hydrogen ion concentration of the blood during the period of lowered alveolar oxygen to pH 7.2 can, as in experiment 1, be attributed to increased pulmonary ventilation, increased volume flow of blood and decreased oxygenation of the blood. The subsequent large increase in the hydrogen ion concentration of the blood amounting to 0.19 pH is accompanied by an increase in lactic acid content of the blood amounting to 55 mgm. per 100 cc. of blood. Again the changes in carbon dioxde capacity of the blood were not proportionate to the changes in lactic acid content of the blood.

With readministration of room air pulmonary ventilation fell distinctly below normal at gas sample 16. Though oxygen consumption more than doubled, carbon dioxide elimination was reduced to less than one-fifth the elimination in gas sample 14. There was a corresponding drop of the expiratory quotient from 2.03 to 0.40 after which there was a slow return to 0.74 which is still below the preadministration level. This prolonged subnormal expiratory quotient is probably related to the rapid disappearance of lactic acid as indicated in the lactic acid curve. The rapid disappearance of lactic acid is accompanied by a reduction in the hydrogen ion concentration from pH 7.15 to 7.24. This final concentration is still higher than the normal and corresponds to a relatively low carbon dioxide content of the blood. The disproportionate change in the carbon dioxide capacity and lactic acid content of the blood is again very definite,

The results of experiment 3 differ in a number of points from those of experiments 1 and 2. While pulmonary ventilation was increased by the administration of gaseous mixtures low in oxygen the sudden high initial increase was entirely missing. In its place there was only a slight augmentation which increased progressively up to the very end of lowered alveolar oxygen. Oxygen consumption showed the customary fall at the beginning of administration, also the secondary increase, but the final decrease noted in experiments 1 and 2 was missing. Apparently the animal was reacting very well to the exposure to low oxygen. In observing the curve of carbon dioxide elimination it is interesting to note that despite the small initially increased ventilation, the early maximum carbon dioxide elimination still obtained and as ventilation increased carbon dioxide elimination decreased as it did in the other experiments. Another striking difference in results is the very small increase in blood lactic acid. This, along with the low initial value, is indicative of a general vigorous condition of the animal. The changes in blood carbon dioxide capacity are also worthy of note. First the carbon dioxide capacity decreased out of proportion to the increase in blood lactic acid and then rapidly increased to supernormal values despite the fact that lactic acid was increasing in the blood. This peculiar phenomenon, though not as pronounced, is clearly evident in experiment 1 and indications of it are also seen in experiment 2. The results are suggestive of an outpouring of base from some carbonate depot such as the bones or the outpouring of red blood cells from the spleen as protective measures against acid tendencies of anoxemia. It will be seen that the blood turned distinctly alkaline in this experiment and instead of showing the usual tendency of increasing acidity of experiments 1 and 2 the blood continued more alkaline throughout low oxygen exposure. To be sure, this well maintained alkalinity may have been due to the increasing pulmonary ventilation as well as to the addition of base to the blood.

When a dog is subjected to an atmosphere of lowered oxygen pressure various changes which occur may conceivably be adjustments conducive to the well being of the organism. Perhaps the outstanding of these are increased circulation and ventilation of the blood. The first is usually associated with a faster rate of heart beat and ejection of blood against a higher pressure. The second is accompanied by a greater movement of air in and out of the respiratory passages, a warming of the greater volume of air, the saturation of this greater volume of air with water vapor and the liberation of a greater amount of carbon dioxide from the blood into the alveolar air. All of these processes are energy consuming. If a continuation of the normal basal rate of dissipation of energy is essential for the continuance of normal basal function it is of interest to determine, if possible, to what extent the demands for energy, basal and superbasal are met under conditions of oxygen stress.

Some of these energy relationships are summarized in table 1, where all values are represented in equivalents of energy in terms of oxidations in cubic centimeters of oxygen (1 cc. of oxygen = 4.8 calories). Each experiment is divided into three periods, the period of low oxygen, the period of recovery and the combined period of low oxygen and recovery. The first line gives the expected basal oxygen consumption assuming a continuation of the basal rate during the preceding period of administration of room air. A comparison of the expected and the observed oxygen consumption during the period of lowered alveolar oxygen shows a small balance in favor of excess oxygen consumption above the basal requirements, but much too small to meet any substantial demands for circulatory and respiratory adjustments. On the other hand, without circulatory and respiratory adjustments oxygen consumption would undoubtedly have fallen far below normal as has been established in our earlier experiments in which artificial ventilation of uniform volume was administered (Gesell, Krueger, Gorham, and Bernthal, 1930). During the period of recovery however oxidations increased and the oxygen balance was definitely in favor of superbasal oxidation to the extent of 47.6, 41.8, and 24.5 cc. For the combined periods the balance was in each case slightly larger—47.9, 43.6, and 28.3 cc. Assuming that the energy equivalent of this excess

TABLE 1

Energy relationships

	EXPERIMENT 1			EXPERIMENT 2			EXPERIMENT 3		
	Low O_2 G. S. 4–11	Recovery G. S. 12–18	Low O_2 and recovery G. S. 4–18	Low O_2 G. S. 4–14	Recovery G. S. 15–22	Low O_2 and recovery G. S. 4–22	Low O_2 G. S. 4–10	Recovery G. S. 10–17	Low O_2 and recovery G. S. 4–17
1. Basal O_2 consumption	151.2	260.9	412.1	270.1	303.2	573.3	134.4	226.3	360.7
2. Observed O_2 consumption	151.5	308.5	460.0	271.9	345.0	616.9	138.2	250.9	389.1
3. Oxygen balance	+0.3	47.6	47.9	+1.8	+41.8	+43.6	+3.8	+24.5	+28.3
4. Anaerobic energy	+28.9	−19.3	+9.6	+40.2	−22.0	+18.2	+10.7	−8.5	+2.2
5. (3) + (4)	+29.2	+28.3	+57.5	+42.0	+19.8	+61.8	+14.5	+16.0	+30.5
6. O_2 work (ventilation)	−0.3	−0.1	−0.4	−0.4	−0.0	−0.5	−0.1	−0.0	−0.1
7. O_2 warming air	−5.8	−1.5	−7.3	−7.2	−0.8	−8.0	−3.7	−0.6	−4.3
8. O_2 saturating air	−22.4	−5.7	−28.1	−27.8	−3.2	−31.0	−14.5	−2.4	−16.9
9. O_2 CO_2 liberation	−11.9	+3.5	−8.4	−13.1	+5.7	−7.4	−8.3	+3.9	−4.4
10. (6) + (7) + (8) + (9)	−40.4	−3.8	−44.2	−48.5	+1.7	−46.9	−26.6	+0.9	−25.7
11. (5) + (10)	−11.2	+24.5	+13.3	−6.5	+21.5	+14.9	−12.1	+16.9	−4.8

oxidation is available for superbasal activities of adjustment and recovery we have added, whether correctly or not we do not know, the anaerobic energy dissipated as a result of liberation of lactic acid which brings the total extra dissipated energy above the basal requirements in terms of oxygen to 57.5, 61.8, and 30.5 cc.

The computed energy required to meet the various altered conditions follow next. Accepting Rohrer's (1925) formula ($W = 0.6\ L^2$ kgm. $= 0.003^-\ L^2$ cc. of O_2 per minute where W is the energy equivalent of the work and L the minute volume) for determining the work required to move the extra air of superbasal ventilation the oxygen requirements are seen to be very small—about 0.4, 0.5, and 0.1 cc. per kilogram hour respectively. (The computations are based on a uniform respiratory rate of 15 per minute.) The energy required for warming the air of superbasal ventilation is greater. Assuming a change in temperature from inspired air at 20°C. to expired air at 32°C. (these temperatures were not followed during the experiments) the energy equivalent of warming the air is 7.3, 8.0 and 4.3 cc. The energy requirements for the heat of liberation of the superbasal carbon dioxide from the blood into the alveolar air are of a similar magnitude, 8.4, 7.4, and 4.4 cc. of oxygen respectively. ($NaHCO_3 + HP$-$NaP + H_2O + CO_2 +$ 10,850 cal. P represents protein anions. Hence the movement of 1 cc. of carbon dioxide into or out of the body involves 0.484 calory or the energy equivalent of 0.101 cc. of oxygen. Derived from the equations of Meyerhof, 1925.) Saturation of the air with water vapor appears to be of the most significant item of all the superbasal requirements. Computing for a saturation from 50 per cent at 20°C. to 100 per cent at 32°C. (Liljestrand and Sahlstadt, 1924) 28.1, 31.0, and 16.9 cc. of oxygen are required for supplying the energy to saturate the air of superventilation. Adding the equivalents of the work of ventilation, of warming and saturating the air and of liberating carbon dioxide from solution it is seen that the total falls somewhat short of total superbasal dissipation of oxidative and anaerobic energy. A further reduction of the balance is conceivable as a result of expenditure of energy for chemical adjustments such as the secretion of the adrenal gland leading to facilitation of oxidations and also expenditure of energy for the repair of cellular damage done during the period of impaired oxidations.

The excess of energy dissipated (aerobic and anaerobic for the duration of the periods studied) was greatest during the period of lowered alveolar oxygen—approximately 19.2 per cent, 15.4 per cent and 10.5 per cent respectively of the basal consumption. For the periods of recovery it was 9.1 per cent, 5.7 per cent, and 6.3 per cent respectively. The average figures for the combined periods were 12.5 per cent, 10.0 per cent, and 7.6 per cent. Attention is called to the fact that in no case had the oxygen consumption returned to the basal level at the end of the observed recovery period

suggesting, that the energy equivalent of cellular repair to membranes, etc., may be a large factor in the final energy balance of perfectly restored tissue. Increased permeability of cell membranes which is known to occur as a result of impaired oxidations supports this view.

In the graphs and preceding description of results the formation and disappearance of lactic acid have been briefly considered in relation to oxygen consumption, carbon dioxide elimination, expiratory quotient, carbon dioxide capacity of the blood and total dissipation of energy. In the following table other relationships are recorded throwing light on the general problem of the significance of lactic acid as a source of food, of energy and a means of acid base control. It seemed desirable to learn of the distribution of lactic acid between blood plasma and tissue cells during normal basal metabolism and to compare this distribution with that at the end of a period of administration of low oxygen mixtures during which blood lactic acid is known to increase. Lactic acid determinations were made on whole blood, blood plasma, blood corpuscles, striated muscle and testicles by the method of Friedemann, Cotonio, and Shaffer (1927). An hour before taking blood and tissue samples symmetrical extensor muscles were dissected free of neighboring tissue in both hind legs to permit a subsequent rapid removal. The nerve and blood supplies were left intact and the skin was closed over the field to approximate normal conditions. Similar preparations were made for rapid removal of the testicles by freeing the testicles and pedicles from the surrounding tissue. When conditions of the animal were apparently uniform, as indicated by the steady head of blood pressure and uniform volume of ventilation, an arterial blood sample was drawn. Then two combination scissors and clamps were carefully slipped under the ends of one of the extensor muscles. At a signal, two operators simultaneously cut and clamped their respective ends of the muscle and the third operator dropped the intervening muscle in liquid air. With smooth technique, the muscle was dropped into the liquid air within one second after the signal. Shortly after the muscle sample was taken the testicle was removed. If sensory stimulation occurred as a result of excision, time was allowed for reëstablishment of more constant conditions, then low oxygen mixtures were administered. At the very end of this period, three similar samples of blood, muscle and testicle were taken which was immediately followed by readministration of room air.

The tissues were kept in liquid air during the experiment and then sectioned in the frozen condition with a microtome. The success of sectioning depends on a firm grip on the tissue and thorough freezing. An adjustable clamp was devised to hold the muscle and testicle and the whole procedure was carried out in a large refrigerator. Four to six samples of approximately 1 gram each were obtained from each muscle and testicle. By occasional cooling of the tissue and clamp in liquid air, easily manipulated

frozen sections were obtained which were dropped into weighted amounts of fixing solutions as they were cut. The temperature and thinness of the section and the temperature of the fixing solution would seem to offer ideal conditions for a minimum formation of lactic acid in the excised tissue as well as for establishment of complete equilibrium between tissue and fixing fluid.

In experiment 1 (see table 2) the basal values for whole blood, plasma, corpuscles, muscle and testicle were 16.5, 20.9, 13.4, 41.0, and 11.0 mgm. per 100 grams. At the end of the period of low oxygen these increased to 54.5, 75.3, 41.7, 55.0, and 19.0 mgm. respectively. The largest increase amounting to 54.4 mgm. occurred in the plasma. The corpuscular content increased 28.3 mgm., the muscle content increased 14.0 mgm., and the testicle content increased 8 mgm. Looking upon muscle as the probable

TABLE 2

Distribution of lactic acid before and after anoxemia

EXPERIMENT		BLOOD LACTIC ACID	PLASMA LACTIC ACID	CORPUSCLE LACTIC ACID	MUSCLE LACTIC ACID	TESTICLE LACTIC ACID
		mgm./100 cc.	*mgm./100 cc.*	*mgm./100 cc.*	*mgm./100 grams*	*mgm./100 grams*
1	Basal	16.5	20.9	13.4	41	11
	After 6.09% O_2	54.5	75.3	41.7	55	19
	Increase	38	54.4	28.3	14	8
2	Basal	60			54	31
	Low O_2 (5.69%)	120			101	75
	Increase	60			47	44
3	Basal	17	16.2	13.7	30	12
	After 7.24% O_2	31.9	43.6	28.1	43	19
	Increase	14.9	27.4	14.4	13	7

primary source of lactic acid, it is of interest that the muscle increase should be lower than that of the plasma which may be looked upon merely as a vehicle of lactic acid. Obviously a significant redistribution of lactic acid has occurred.

Basal concentrations show a higher value for plasma than for corpuscles, the difference amounting to 7.5 mgm. per 100 cc. of blood. This is in agreement with the findings of Hill, Long and Lupton (1924); Gesell, Krueger, Gorham, and Bernthal (1930); and Eggleton and Evans (1930). A similar excess of plasma content over cell content (9.9 mgm. per 100 G.) is seen for the testicle. The muscle content is, however, decidedly higher than that of the plasma suggesting that lactic acid moves from the primary source of high concentration to the blood along a positive gradient. In a similar way, it might be inferred that lactic acid is diffusing into the

blood corpuscle and into the cells of the testicle along a positive gradient. The relatively low values of testicular content might conceivably be due to a slow diffusion of lactic acid into the cells and a relatively rapid consumption after it has entered. In the case of the corpuscles a relatively rapid consumption of lactic acid most probably does not occur. The distribution of lactic acid between plasma and corpuscles perhaps is, therefore, best explained by the Donnan membrane equilibrium.

That various tissues may remove lactic acid from the blood is now well established—striated muscle, Barr and Himwich (1923); cardiac muscle, Himwich, Koskoff, and Nahum (1930); brain, McGinty (1929); liver, Himwich, Koskoff, and Nahum (1930); McClure (1929); and Eggleton and Evans (1930). The experiments of McClure (1929), showing an increased difference in the hydrogen ion concentration between portal and hepatic blood following an increase in blood lactic acid produced by intravenous injection of sodium cyanide, indicate augmented removal of lactic acid from the blood stream. The liver thus tends, not only to continue a positive diffusion gradient for lactic acid from muscle to blood, but to con-

TABLE 3

Order of lactic acid concentration

EXPERIMENT	BEFORE OXYGEN LACK					AFTER OXYGEN LACK				
1	M	P	B	C	T	P	M	B	C	T
2	B	M			T	B	M			T
3	M	B	P	C	T	P	M	B	C	T

tribute base to the blood by conversion of lactic acid into glycogen. The liver, consequently, acts in a very important way to control the acid-base equilibrium of the body. A continuous removal of lactic acid from the blood by various organs and tissues might, therefore, influence the general distribution of lactic acid and account for the lower concentration of lactic acid in the arterial blood than in muscle as found in these experiments.

But since lactic acid appears to be a product of anerobic metabolism of all mammalian tissues it is reasonable to assume that with major anoxemia all tissues would be forming lactic acid faster than they are consuming it. The lowering of the arterial level below muscle level would then come to an end. McGinty's (1929) direct experiments on the brain show that during minor anoxemia the brain continues to absorb lactic acid from the arterial blood stream, but when impairment of oxidations increases, the brain, like muscle, contributes lactic acid to the blood. To what extent the contribution of lactic acid to the blood by the tissues in general and its removal by the liver counteract each other during varying degrees of impairment of oxidations is not known. It will be seen that lactic acid increases in

amount in the blood plasma with the progress of anoxemia and actually increases to a concentration considerably above that of muscle. This higher concentration, therefore, raises two significant questions. Is some tissue other than muscle contributing lactic acid to the blood stream at a greater rate than muscle and thereby raising the blood concentration, or has the failure of adequate removal of lactic acid from the blood by the liver and other tissues simply brought into evidence membrane activity leading to a demonstrable partition of lactic acid between the cell and surrounding fluids? Accepting the view that the hydrogen ion concentration of the interior of cells is greater than that of the environment, the Donnan membrane equilibrium calls for a lower value of lactic acid in the red blood corpuscle, muscle and testicle as compared with blood plasma. Assuming another possibility that the hydrogen ion concentration of the mammalian tissue cells decreases to a smaller extent than that of the blood plasma and tissue fluids with the progress of anoxemia (see below) the Donnan membrane equilibrium becomes of increased general importance in explaining the distribution of lactic acid during lowered alveolar oxygen.

In experiment 2 lactic acid also increased in the blood, muscle and testicle as a result of lack of oxygen. Separate determinations of plasma and corpuscular lactic acid content are missing, but since there were no other exceptions to the rule (eight experiments) and since it is a fact well established by other workers it is assumed that in this experiment, too, the plasma content was higher than the corpuscular content. Comparison of the concentration of whole blood with that of muscle during basal conditions shows the blood content to be higher than that of muscle. This difference increased with anoxemia. In experiment 3 the results are more comparable to those of experiment 1. During basal conditions the muscle content was higher than the plasma content and the corpuscle and testicle contents were lower. During anoxemia the plasma content increased most and just exceeded the muscle content.

By adding the energy liberated from the formation of lactic acid to the caloric equivalent of oxygen consumed, a so-called total dissipation of energy was obtained during the period of oxygen lack, and by subtracting the energy absorbed by reconversion of lactic acid to the precursor state from the caloric equivalent of oxygen consumed the total energy dissipated during the period of recovery was obtained. Since there is both an increased formation of lactic acid and an increased oxygen consumption during the period of lowered alveolar oxygen the increase in dissipation of energy is relatively larger than during the period of recovery where the absorption of energy represented by the lactic acid shift is subtracted from the oxidative energy.

The mechanisms controlling the dissipation of energy may be numerous, but it would appear that changing concentration of lactic acid could influ-

ence oxidations in two important ways. By increasing the hydrogen ion concentration of the tissues, lactic acid might tend to decrease oxidations and by increasing the amount of oxidizable material it might tend to augment oxidation. Since carbon dioxide is blown off in increased amounts during anoxemia while lactic acid is accumulating the acid effects of reduction in buffer base are counteracted. The synchronous accumulation of lactic acid and reduction in carbon dioxide, permitting oxidations to continue at a relatively high rate, then become a self corrective joint measure maintaining dissipation of energy at an adequate level. Just to what extent the blowing off of carbon dioxide opposes the acid effect of accumulation of fixed acid and reduction of buffer base cannot be accurately determined with the data at hand, but since the question is of importance in the control of respiration and ventilation we have attempted a tentative analysis. A refinement of procedure and conclusions must await later experiments.

For a rough approximation of the gross change in tissue acidity throughout the body as a whole, it was assumed that all the tissues have a similar metabolism, and are similarly affected by lack of oxygen, that the circulation is uniform throughout and that changes in the carbon dioxide pressure in the tissues are proportional to the changes in carbon dioxide pressure in the expired air. Obviously these simple conditions do not obtain. Nevertheless they are assumed, as a working basis, to conjecture the possible changes in hydrogen ion concentration of the tissues.

Employing Hasselbalch's equation and using for the respective values of the variables during basal conditions the subscript (B) and experimental conditions the subscript (E) we have

$$\mathrm{pH}_B = K + \mathrm{Log}\,\frac{(\mathrm{Total\ CO_2})_B - (\mathrm{Free\ CO_2})_B}{(\mathrm{Free\ CO_2})_B}$$

$$\mathrm{pH}_E = K + \mathrm{Log}\,\frac{(\mathrm{Total\ CO_2})_E - (\mathrm{Free\ CO_2})_E}{(\mathrm{Free\ CO_2})_E}$$

The change in pH is given by

$$\mathrm{pH} = \mathrm{pH}_B - \mathrm{pH}_E = \mathrm{Log}\,\frac{(\mathrm{Total\ CO_2})_B - (\mathrm{Free\ CO_2})_B}{(\mathrm{Free\ CO_2})_B} - \mathrm{Log}\,\frac{(\mathrm{Total\ CO_2})_E - (\mathrm{Free\ CO_2})_E}{(\mathrm{Free\ CO_2})_E}$$

Assuming an average basal total carbon dioxide content of the tissue of 24 cc. the corresponding value at another time may be obtained by subtracting the non-oxidative CO_2 which appears during the interval. The

partial pressure of carbon dioxide in the tissues during basal condition is assumed to be 50 mm. Hg.

With these values the changes in pH will be:

$$\mathrm{pH} = \mathrm{pH}_B - \mathrm{pH}_E = \mathrm{Log}\,\frac{24 - (0.0684 \times 50)}{(0.0684 \times 50)} - \mathrm{Log}\,\frac{(24 - \mathrm{N}) - (0.0684 \times 50)F}{(0.0684 \times 50)F}.$$

where N is the average change in carbon dioxide content or the non-oxidative carbon dioxide eliminated between the two states and F is the ratio of the carbon dioxide in the expired air during rest to that during the administration of low oxygen. The changes in pH computed in this way are tabulated and presented in table 4. Negative values indicate a greater alkalinity of the tissues at the end of the period of oxygen lack. The computations (see table 4) therefore suggest that the alkaline effect of greater elimination of carbon dioxide more than compensates the acid effects of

TABLE 4

Calculated changes in tissue acidity

1	2	3	4	5	6
CO_2 EQUIVALENT OF INCREASE IN LACTIC ACID	NON-OXIDATIVE CARBON DIOXIDE	PARTIAL PRESSURE OF CARBON DIOXIDE IN EXPIRED AIR		pH OF TISSUES, CALCULATED	pH OF BLOOD, OBSERVED
		B	E		
cc./kgm.	*cc./kgm.*	*mm.* Hg	*mm.* Hg		
3.50	11.83	30.0	15.4	−0.00	−0.10
11.75	12.16	21.7	13.0	−0.10	−0.00
			18.4	−0.29	−0.05
3.25	7.93	34.2	14.0	+0.24	+0.21

accumulation of lactic acid in experiments 1 and 3 producing a resultant decreased hydrogen ion concentration of the tissues. In experiment 2, pH changes are given for gas sample 13 when the carbon dioxide pressure was 13 mm. Hg and for gas sample 14 when it was 18.4 mm. Hg. At this stage the animal was failing very rapidly, lactic acid was accumulating at a high rate, both blood pressure and ventilation were falling off. It is therefore highly probable that the computed increase in tissue hydrogen ion concentration above the normal is grossly correct. Note also that the pH of the blood was at the same time changing in a similar direction.

Though these experiments indicate some of the chemical processes which are at work supporting oxidations and the dissipation of energy they do not indicate specifically the chemical mechanisms controlling ventilation. To be sure, our calculated decrease in the gross hydrogen ion concentration of the animal might be interpreted as establishing a decrease in hydrogen ion concentration of all of the cells of the body including the respiratory neurones which control the respiratory act. But this by no means follows.

For the present all that can be said is that pulmonary ventilation is stimulated by some effect or several effects of lack of oxygen. The effects of lack of oxygen must be numerous— changes in concentration of the chemical constituents of the cell, changes in concentration of ions including the lactate, bicarbonate and hydrogen ions, changes in oxidation and reduction potentials, changes in the structural state of neuro-membranes including permeability, etc.

If increased ventilation from lowered alveolar oxygen is due to some effect other than increased hydrogen ion concentration, augmented ventilation might continue at a magnitude sufficient to turn the hydrogen ion concentration of the respiratory neurones to a subnormal level without necessarily entailing subnormal ventilation. On the other hand, if increased hydrogen ion concentration of the respiratory neurone is the stimulus, then ventilation must stop short of a point which will turn the neurones more alkaline than normal. If it did not, apnea or subnormal ventilation would result. Assuming an acid mechanism of control, it is necessary to conclude that the tissues as a whole were turned more alkaline than normal by virtue of an increase in the hydrogen ion concentration of the neurones controlling ventilation. Either view is compatible with the findings of our present experiments.

While the experiments showing rapid formation of lactic acid in the brain under anaerobic conditions (McGinty and Gesell, 1925) and reduced absorption of lactic acid from the blood or increased formation with graded impairment of oxidations (McGinty, 1929) and reduced carbon dioxide capacity with impaired oxidations support the possibility of an acid mechanism of control, they are not interpreted as establishing the fact. Neither are our present experiments suggesting that the brain as a whole may have turned less acid than normal during lowered alveolar oxygen interpreted to mean that the respiratory neurones have experienced the same directional change. We are inclined to believe that positive conclusions regarding the hydrogen ion concentration of the respiratory neurones during a response to lowered oxygen pressure are fraught with serious difficulty.

In this connection we are reminded of the recent report of Myerson, Loman, Edwards and Dill (1931) in which they state

> Our observations are more in line with those of Gesell and associates (1929). He has come to the conclusion that hydrogen ion concentration can no longer be considered the universal stimulus to respiration but that the metabolic processes in the center itself must be considered. As pointed out frequently by Henderson (1928) it is illogical to assign unique functions to a single variable such as $(H)^+$ when variations in it are necessarily associated with changes in concentration of other ions.
>
> Our results fit in very well with those of Cullen and associates (in press) who have shown that when patients with heart disease are exercised a large increase in pulmonary ventilation takes place with no increase in acidity of blood passing through the

brain. So far as we can judge the increase in pulmonary ventilation which occurs in anoxemia is associated with a normal oxygen supply to the brain and with more alkaline reaction than normal in blood leaving the brain.

While we wish to maintain our position that the control of ventilation is undoubtedly a multiplex phenomenon even to the extent that factors accessory to the hydrogen ion may under certain conditions be important enough to permit increased ventilation with subnormal hydrogen ion concentration of the respiratory neurones, we find difficulty in agreeing with the inference that the *respiratory neurones* do turn less acid during anoxemia. There are a number of points which need careful consideration in this connection to determine definitely whether an acid or alkaline effect occurs.

The mixed cerebral blood sample may not contain the outflow from the respiratory center, or if it does, the blood coming from the center may be so small in amount compared with the total cerebral flow that even a gross change in its composition would remain unregistered in the analysis of the total mixed sample. It is not at all inconceivable that the actual effective portion of the respiratory neurone arc responsive to chemical excitation may be only 0.001 to 0.0001 or even less of the total brain bulk.

The rate and nature of acid metabolism of the respiratory center cannot be assumed to be the same as that of the brain as a whole. Just as Haldi (1931) has shown that the rate and amount of lactic acid formed in isolated tissues under anaerobic conditions varies with the tissue so Haldi, Ward and Woo (1927) showed that the percentage content of lactic acid varies in different parts of the brain. More than twice as much lactic acid is formed in a unit weight of medulla tissue than in the tissue of the cerebral hemispheres.

The facilities for supply of oxygen and removal of acid must vary throughout the brain, for the distribution of capillaries is known to vary from one region to another (Craigie, 1926). Whether a cell or a block of tissue nourished by a given set of capillaries will turn more acid or less acid during lowered alveolar oxygen will depend upon the rate and nature of the acid metabolism, the pressures of oxygen, of carbon dioxide, and of lactic acid inside and outside of the cell, the distances of diffusion and the relative rates of diffusion of oxygen and carbon dioxide and lactic acid, and the buffering value of the tissue. If the requirements for oxygen are high and distances of diffusion are great, anaerobic metabolism with its consequent formation of lactic acid is likely to occur. A high lactic acid formation per unit of oxygen lack and a low rate of diffusion of lactic acid provides additional conditions for increased hydrogen ion concentration.

Increased functional activity of the respiratory neurones and consequent increased requirements for oxygen and removal of acids are other factors to be considered. Garrey (1920) has shown increased formation of acid in activated neurones. Fenn (1928) has shown the same for nerve fibers.

The problem of determining whether or not the respiratory neurones turn acid with lowered alveolar oxygen is obviously a difficult one. A direct experimental approach such as isolation or cannulation of a vein coming from the effective respiratory neurones seems for the present virtually impossible. New approaches must be made. One of us (Krueger, to be published) has attempted to interpret in terms of hydrogen ion concentration the conditions obtaining in the brain with respect to the requirements of oxygen and the facilities for diffusion of oxygen, carbon dioxide and lactic acid. Computations, to be sure, based on insufficient data confirm the suspicion that the hydrogen ion concentration of the larger brain cells or collection of brain cells without individual capillary supply may increase with lowered alveolar oxygen while the smaller cells suffer a decrease. We have already called attention to the computations of the present paper indicating that the body as a whole turns more alkaline with lowered alveolar oxygen.

In addition to these considerations the problem is involved from another important angle for it is no longer possible to consider the respiratory center as the only seat of chemical control.

The important experiments of Heymans (1931) demonstrated that the carotid sinus and the afferent vagus nerve endings are sensitive to lowered oxygen pressure, sodium cyanide and sodium sulphide. These results have been confirmed by Owen and Gesell (1931). While finding marked stimulation from local action of cyanide at the sinus Owen and Gesell were also able to demonstrate definite though smaller stimulation from arterial injection after denervation of the sinus and from flooding of the 4th ventricle. Painting of the carotid sinus and the floor of the fourth ventricle with weak sodium sulphide solution had marked and apparently equal stimulating effects. In agreement with Heymans, injections of acid and base were apparently equally effective whether these substances were injected central or peripheral to the sinus.

Of interest are the experiments from this laboratory of Glazer (1929), Winkler (1930), and Gay (1931) which demonstrated changes in excitability of various portions of the reflex arc under several conditions modifying pulmonary ventilation. Winkler showed fluctuations in the reflex response of the tibialis anticus running parallel to fluctuations in ventilation accompanying hemorrhage and reinjection. Gay (1931) showed a similar parallelism with administration of sodium cyanide, sodium sulphide, and gaseous mixtures low in oxygen. On the other hand, administration of carbon dioxide was found to lessen the reflex response. These workers suggested accessory chemical control, peripheral to that in the so-called respiratory center, operating on the peripheral sensory and motor fibers, on the cord and on muscle directly.

Recent experiments, therefore, indicate both central and peripheral

regulative mechanisms seemingly of varying kind and significance in the control of pulmonary ventilation. Whether the less certain effects of central action of cyanide as compared with sinus stimulation are attributable to a resultant of excitation at one point in the respiratory neurone chain and depression at another remains for further study. Why lowered alveolar oxygen augments spinal reflexes and increased alveolar carbon dioxide depresses them also requires further consideration. Regardless of possible interpretations, it is now as essential to determine how chemical changes excite peripherally as it is to determine how they act on the so-called respiratory center. This obviously will require further chemical studies but along with these must go intensive and detailed study of the respiratory nerve impulse at various stations in the complex respiratory reflex arc.

Still ignorant of the fundamentals involved in the control of the respiratory nerve impulses our views regarding the chemical changes which are responsible for the control of ventilation must remain indefinite. While our present data do not seem to be opposed to a significant acid mechanism of control under various conditions it is reminded that (Gesell, 1929)

> the theory in no way proposes that the hydrogen ion is the one and only regulator of respiration for obviously the hydrogen ions are operating on a highly complex and relatively unstable mechanism about which we know very little. For example, the state of oxidation of the neurone membrane may be a contributing factor of control. The position that tissue acidity is the only factor controlling ventilation is thus untenable. To quote from my last review (1925) "It should be recalled that the regulation of respiration in its ultimate analysis may be found to be an electrical phenomenon occurring with the aid of a surface membrane, and changes in composition of the fluids on both sides of that membrane. Whether acid is the only agent which can produce the necessary electrical disturbance involved in the control of respiratory nerve impulses is highly improbable; on the other hand, it may be the agent most employed in the body."

SUMMARY AND CONCLUSIONS

In a preceding series of papers numerous factors relating to the control of respiration and ventilation were followed simultaneously on individual animals in which ventilation was artificially administered and maintained at a uniform volume.

In the present series of experiments a similar study was made with pulmonary ventilation under the control of the respiratory mechanisms of the body.

We, therefore, compare in unusual detail the essential findings in both series in order to accentuate the effects of controlled ventilation and the conditions under which augmented ventilation occurs.

During uniform ventilation, administration of gaseous mixtures low in oxygen invariably decreased oxygen consumption and readministration of

room air temporarily increased oxygen consumption considerably above the normal level. The excess oxygen consumption as a rule was less than the preceding shortage.

With ventilation under normal control oxygen consumption increased, both during the period of lowered alveolar oxygen and during the subsequent period of recovery. The increase in oxygen consumption during lowered alveolar oxygen was temporary and relatively small.

Carbon dioxide elimination during constant ventilation changed much less than the coincident fluctuations in oxygen consumption. In most experiments carbon dioxide elimination decreased, in some experiments it remained virtually unchanged and in others it increased slightly above normal. Readministration of room air almost invariably increased elimination of carbon dioxide above the normal level. In one experiment in which carbon dioxide elimination was greater than normal during lowered alveolar oxygen pressure the primary effect of readministration of room air was a reduction in carbon dioxide elimination.

In contrast to these results, during normally controlled ventilation carbon dioxide elimination increased enormously during the period of lowered alveolar oxygen and decreased below normal with subsequent administration of room air. The increase in elimination during lowered alveolar oxygen was greater than the decrease below normal during recovery. The increased elimination was greatest during the early part of lowered alveolar oxygen and the decreased elimination was greatest during the early part of the period of recovery.

These alterations in gaseous exchange resulted in fluctuations in the expiratory quotient—an increase during the period of administration of gaseous mixtures low in oxygen and a decrease below normal following the readministration of room air. This held for both series of experiments—uniform and variable ventilation. The greatest increase in the expiratory quotient obtained during the initial period of oxygen lack and the greatest decrease during the early part of the recovery. The respective increases and decreases in the expiratory quotients were greater in the experiments in which ventilation was normally adjusted.

Lactic acid accumulated in the arterial blood during the period of lowered alveolar oxygen and decreased or showed a tendency toward a decrease with readministration of room air. This held for both series of experiments. During lowered alveolar oxygen the increase was greater in the experiments with uniform artificial ventilation. The decrease during recovery was more pronounced in the experiments in which ventilation was under normal control. In two experiments (nos. 2 and 3) with normally adjusted ventilation, the increase in lactic acid during lowered alveolar oxygen temporarily gave way to a short period of decreasing lactic acid content.

Administration of gaseous mixtures low in oxygen during constant ven-

tilation was accompanied by a decrease in the hydrogen ion concentration of arterial blood as measured with the quinhydrone electrode. This occurred despite the accumulation of lactic acid in the blood and the reduction of carbon dioxide elimination from the lungs. Readministration of room air produced a temporary increase in the hydrogen ion concentration followed by a return towards the normal level. The same directional changes occurred during normally controlled ventilation, but the decrease in hydrogen ion concentration during lowered alveolar oxygen was greater and the increase above normal with readministration of room air was less. Since the decrease in hydrogen ion concentration of the arterial blood during lowered alveolar oxygen and uniform ventilation was associated with an increase in lactic acid and a decreased elimination of carbon dioxide, it was attributed primarily to the reduced state of the hemoglobin. The increase in hydrogen ion concentration above normal occurring with readministration of room air was attributed to the lowered buffer base of the blood from the accumulation of fixed acid and to the increased carbon dioxide pressure resulting from suddenly augmented oxidations. The greater decrease in hydrogen ion concentration during lowered alveolar oxygen with normally controlled ventilation was attributed to the freer blowing off of carbon dioxide from the blood and tissues. The smaller increase in hydrogen ion concentration with readministration of room air was attributed to the greater desaturation of the blood and tissues at the moment augmented formation of carbon dioxide was reëstablished.

The increase in the expiratory quotient above normal during lowered alveolar oxygen and uniform pulmonary ventilation was attributed to the formation of lactic acid and its reaction with bicarbonate, and to increased volume flow of blood. To this should be added the greater carrying power of the less perfectly oxidized blood assisting a freer elimination of carbon dioxide (Henderson, 1928). The greater increase in the expiratory quotient during normally controlled ventilation was attributed to the freer elimination of carbon dioxide resulting from greater ventilation. The decrease in the expiratory quotient with readministration of room air during uniform ventilation was attributed to a liberation of free base on reversion of the lactic acid cycle and to a reduction in volume flow of blood. The greater decrease in the expiratory quotient during normally controlled ventilation when room air is readministered was attributed to the sudden reduction in ventilation, to the desaturated condition of the tissues and blood and possibly to a more perfect reversal of the lactic acid cycle leading to a freer liberation of base. The changes in the expiratory quotient are thus attributed primarily to fluctuations in non-oxidative carbon dioxide—eliminated and retained.

In the present experiments, in which ventilation was normally controlled, the distribution of lactic acid between the blood plasma and the red blood

cell, striated muscle cell and the cells of the testicle was determined. In two experiments out of three, during administration of room air, the lactic acid content was highest in muscle, lowest in the testicle, and second lowest in the red blood cell. Plasma and blood concentrations were variable. During lack of oxygen there was a differential increase in lactic acid. Beginning with the highest the new order of concentration was plasma, muscle, whole blood, red blood cell, and testicle.

It was tentatively suggested that muscle is the primary source of blood lactic acid; that blood lactic acid may pass into various tissues where it in turn may be consumed or deposited as a lactic acid precursor; that lactic acid may pass into the blood stream along a positive or negative diffusion gradient; that the lower concentration of lactic acid in arterial blood plasma as compared with striated muscle under basal conditions may be due to the removal of lactic acid from the blood stream by the liver, heart, brain and other organs; that a failure to remove lactic acid from the blood stream during severe oxygen lack results in its accumulation in the blood and tissues. This accumulation in the blood, it is suggested, permits a demonstrable distribution of lactic acid agreeing with the Donnan membrane equilibrium.

Assuming equal distribution of lactic acid between the blood (whole blood was used) and the tissues, it was concluded that in the uniform ventilation experiments approximately 40 per cent of the lactic acid which was liberated combined with bicarbonate to form carbonic acid. Since the present experiments indicate that lactic acid accumulates in the blood more than in the tissues the amount reacting with bicarbonate may therefore have been somewhat higher.

By adding the caloric equivalents of oxygen consumed to the energy shift accompanying the lactic acid shift, total energy production was computed. With uniform ventilation energy production fell, as a rule, during the period of oxygen lack and invariably rose above the normal level on readministration of room air. When the rate of lactic acid production per unit of oxygen shortage was high the total energy production increased above normal during the period of oxygen lack.

In the experiments in which ventilation was under normal control the total dissipation of energy increased both during the period of administration of gaseous mixtures low in oxygen and during the subsequent period of recovery. The increase in energy dissipation during lowered alveolar oxygen (caloric equivalent of excess oxidations plus energy liberated with the formation of lactic acid) was greater than the increase in energy dissipated during an equal period of recovery (caloric equivalent of excessive oxidations minus the energy absorbed as a result of transformation of lactic acid to its precursor state).

It was concluded that excess energy is required for adjusting the animal to

lowered oxygen pressures and for recovery from the effects of lowered alveolar oxygen. It is suggested that 1, the work of ventilation involved in moving the extra air; 2, the warming of the extra air; 3, the saturation of the extra air with water vapor; 4, the liberation of carbon dioxide from the blood into the alveolar air; 5, the increased cardiac output; 6, the chemical adjustments for augmenting oxidations, and 7, the repair of cellular damage from impaired oxidations may be the primary energy consuming processes. The energy consumed was computed for processes 1, 2, 3 and 4. Of these the saturation of the superbasal air with water vapor represented the greatest expenditure of energy. The sum of the computed energy for processes 1, 2, 3 and 4 was found to be less than the superbasal dissipation of energy for the combined periods of lowered alveolar oxygen and recovery indicating that the repair of cellular damage from acute exposure to lowered oxygen pressures may be a costly expenditure of energy.

Assuming a uniform aerobic and anaerobic metabolism and volume flow of blood throughout the body an approximate computation indicating a decrease in the gross hydrogen ion concentration of the tissues is presented. According to these computations the decrease in hydrogen ion concentration is a result of a lowering of carbon dioxide pressure which more than compensates for the reduction of bicarbonate base by the accumulation of fixed acid.

It is suggested that oxidations are supported during lowered alveolar oxygen by an accumulation of oxidizable material such as lactic acid and by a general reduction in hydrogen ion concentration of the tissues.

Assuming an acid mechanism of control it is necessary to conclude that the tissues as a whole were turned more alkaline than normal during lowered alveolar oxygen by virtue of an increase in the hydrogen ion concentration of some portion of the respiratory neurone chain controlling ventilation. It is pointed out however that the present experiments do not indicate which of several chemical changes resulting from lowered alveolar oxygen are responsible for augmented ventilation.

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A COMPARISON OF THE RESPONSE OF THE ANESTHETIZED DOG TO INTRAVENOUS ADMINISTRATION OF SODIUM CYANIDE DURING UNIFORM ARTIFICIAL VENTILATION AND DURING NORMALLY CONTROLLED VENTILATION WITH ADDITIONAL OBSERVATIONS ON THE EFFECTS OF METHYLENE BLUE

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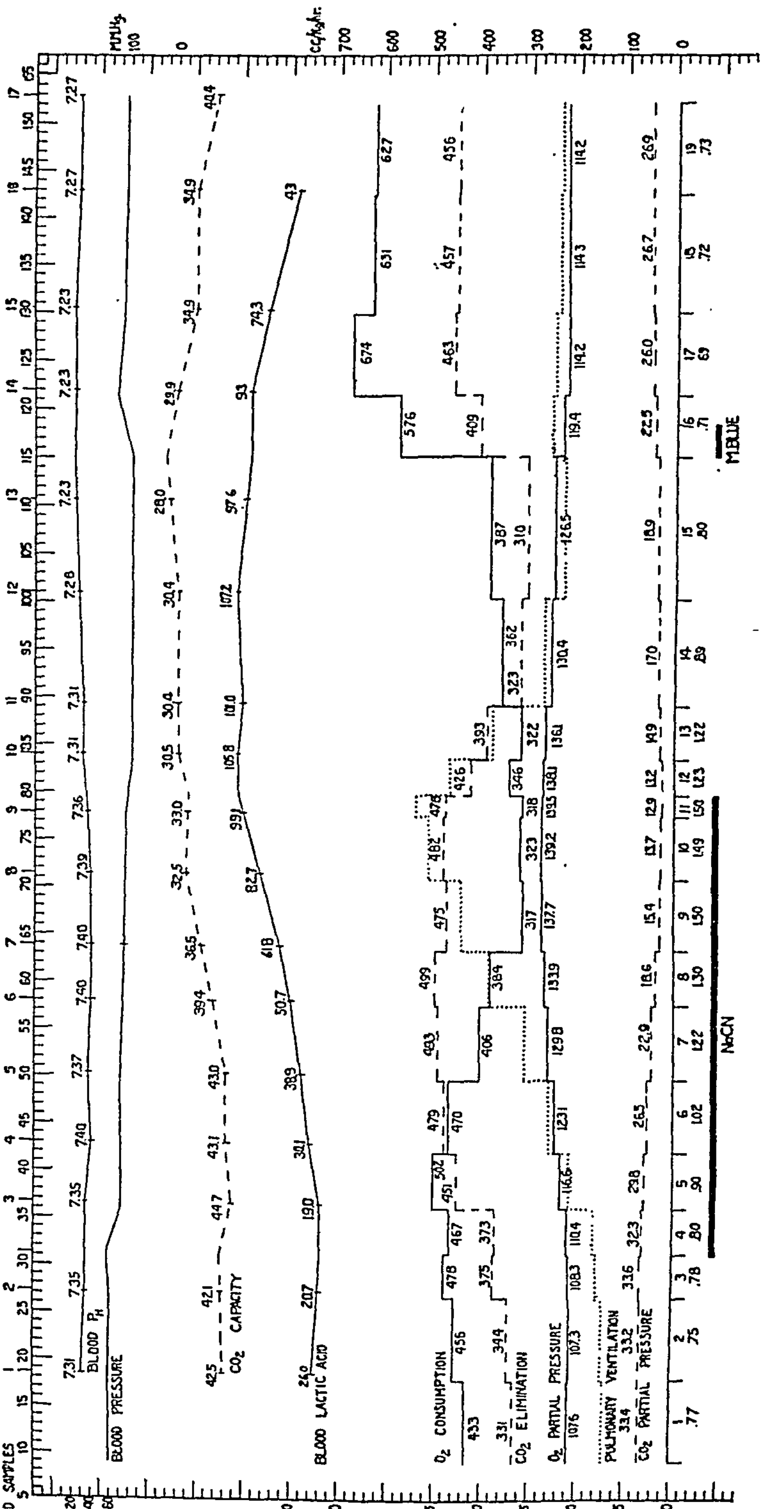
In this series of experiments oxidations were impaired by intravenous injection of 0.004 M. solution of sodium cyanide in isotonic salt solution. Cyanide was injected at a rate sufficient to increase ventilation and decrease oxidations just short of depression of the respiratory and circulatory systems. Continuous injection was accomplished by oil displacement of a cyanide solution contained in a 500 cc. burette sealed above. The oil was delivered to the bottom of the burette and at a pressure of approximately 400 mm. Hg. This relatively high pressure insures a constant rate of injection by minimizing the effects of variations of venous pressure. Allowing oil to rise in drops from an upward pointing dropper permits observation and control of the rate of displacement of the cyanide solution. Due to the accumulative action of cyanide, which not infrequently required a change in rate of injection, irregularity of pulmonary ventilation was occasionally unavoidable.

Four experiments were performed. The results of two of these are presented in graphic form in figures 1 and 2. Experiments 1, 3 and 4 are characterised by even and progressively increasing response to injection. In experiment 2 the effects of injection are more pronounced and the early vigorous response gives way to depression. This in turn is followed by partial recovery at the close of injection as in experiments 1, 3 and 4.

In experiment 1 sodium cyanide was administered for a period of 48 minutes. There was a continuous increase in ventilation from about 7000 cc. per kgm. hour to about 27000 cc. up to the very end of injection. This was accompanied by an increase in oxygen pressure of the expired air from 108.3 mm. to 139.5 mm. The carbon dioxide pressure of the expired air fell coincidentally from 33.6 mm. to 12.9 mm. Oxygen consumption fell from 478 cc. per kgm. hour to 318 cc. and carbon dioxide


BLOOD SAMPLES
TIME
BLOOD P_H
BLOOD PRESSURE
CO_2 CAPACITY
BLOOD LACTIC ACID
O_2 CONSUMPTION
CO_2 ELIMINATION
O_2 PARTIAL PRESSURE
PULMONARY VENTILATION
CO_2 PARTIAL PRESSURE
NaCN
M.BLUE
MM.Hg
cc/kg/hr.

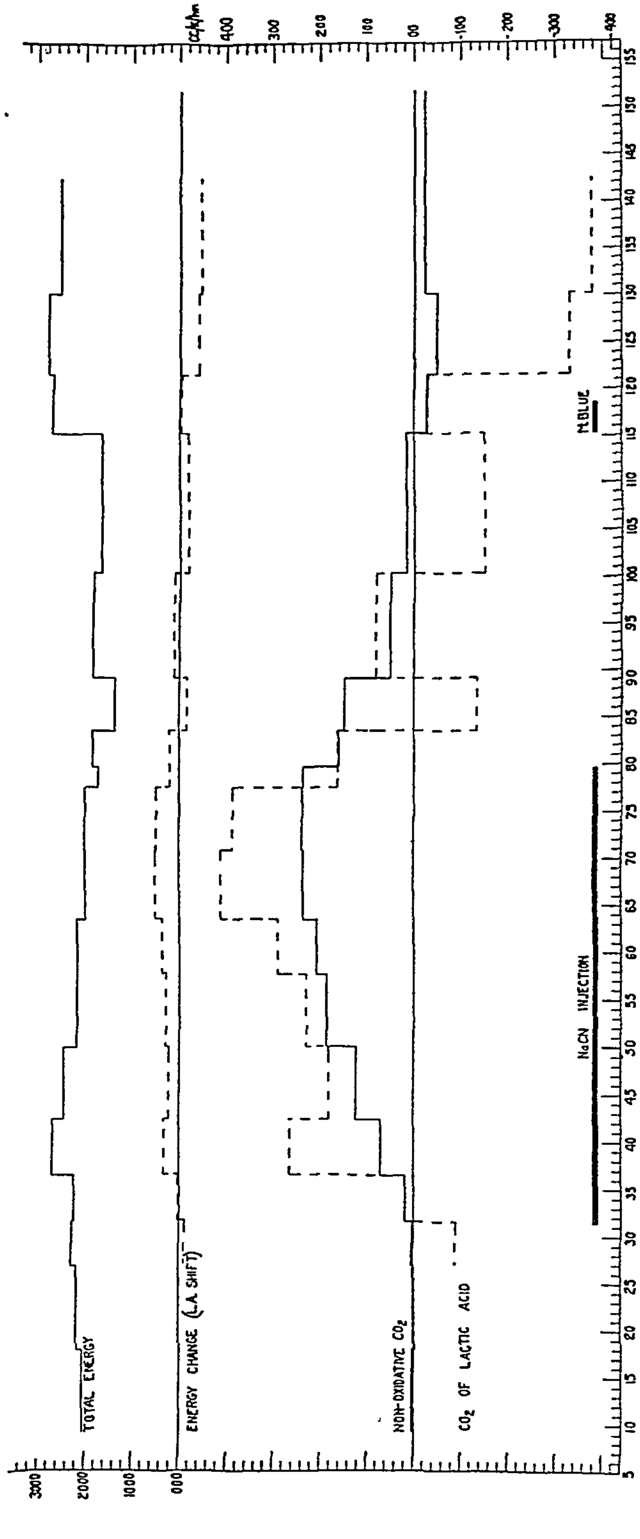


Fig. 1. Experiment 1, June 19, 1930

elimination increased from 375 cc. per kgm. hour to 478 cc. The expiratory quotient rose from 0.78 to 1.50. The blood lactic acid increased from 19.0 mgm. per 100 cc. to 99.1 mgm. This along with the increased ventilation, readily accounts for the increased expiratory quotient. The decrease in blood carbon dioxide was relatively small compared with the increase in blood lactic acid. The hydrogen ion concentration of the blood decreased from pH 7.35 to 7.40, but increased again towards the close of injection to pH 7.36 despite the progressively augmenting ventilation. This increase in hydrogen ion concentration is most probably associated with the rapidly increasing blood lactic acid concentration.

Recovery processes set in immediately at the close of injection and conditions progressively approached those of the preinjection period. Normal recovery processes were later interrupted by intravenous injection of methylene blue. Just prior to methylene blue administration pulmonary ventilation had fallen to approximately 11,500 cc. per kgm. hour. Oxygen consumption had increased from 318 cc. per kgm. hour to 387 cc., the partial pressure of expired oxygen falling in the meantime from 139.5 mm. to 126.5 mm. Carbon dioxide elimination had fallen from 476 cc. per kgm. hour to 310 cc. accompanied by an increase in the partial pressure of expired carbon dioxide from 12.9 mm. to 18.9 mm. The expiratory quotient had fallen from 1.50 to 0.80, which was associated with only a small drop in blood lactic acid from 105.8 mgm. per 100 cc. of blood to 97.6 mgm. The acidity of the blood increased progressively from pH 7.36 to 7.23 which is 0.08 pH more acid than that of the preinjection period.

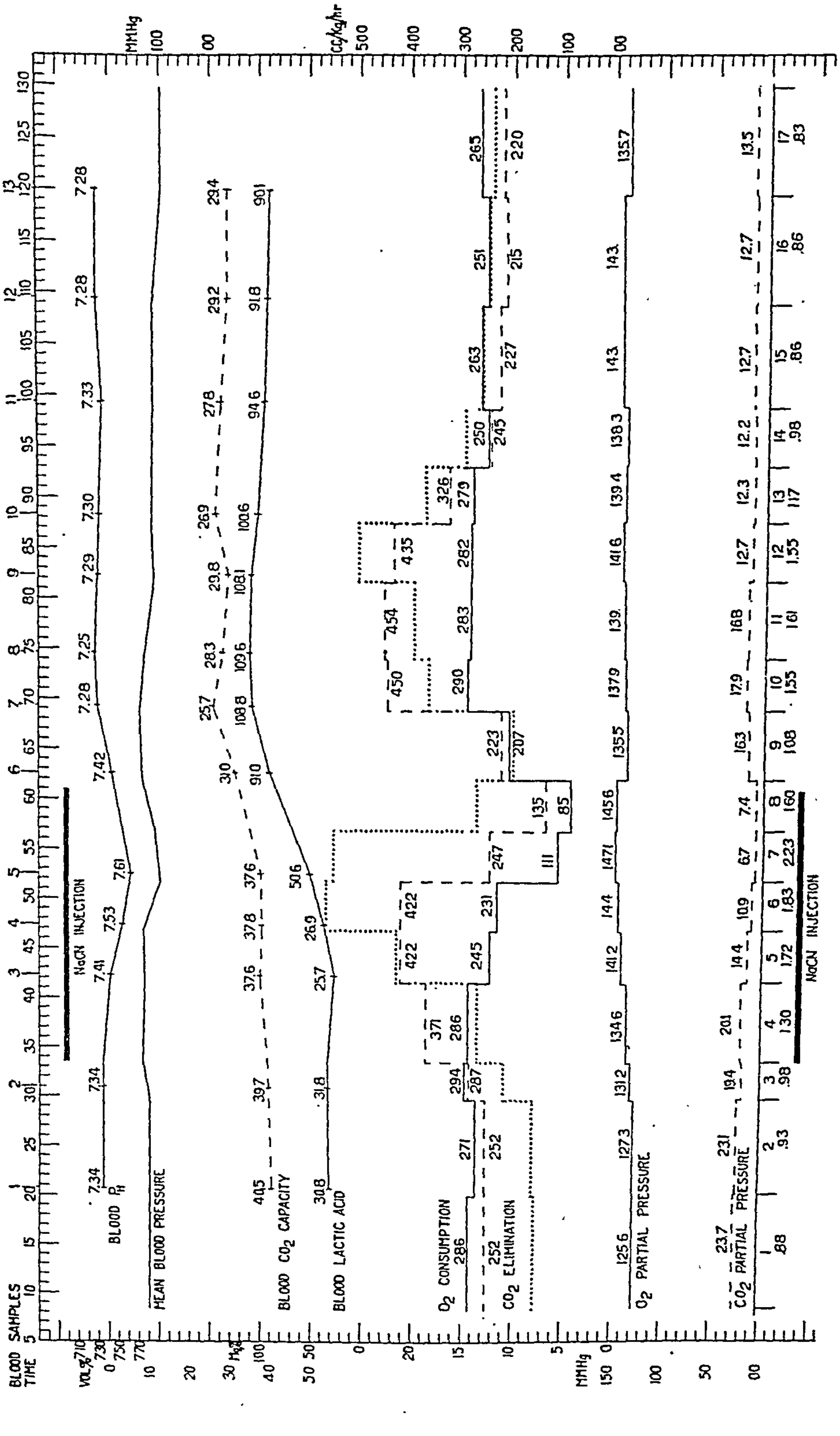
At gas sample 16, during the injection of methylene blue, striking changes occurred, all of which are suggestive of suddenly augmented recovery. Oxygen consumption increased from 387 cc. per kgm. hour to 674 cc. per kgm. hour. Carbon dioxide elimination increased from 310 to 463 cc. per kgm. hour. The partial pressure of expired oxygen decreased from 126.5 to 114.2 mm. Hg. Carbon dioxide partial pressure increased from 18.9 to 26.9 mm. Hg. The blood lactic acid content fell rapidly and the blood carbon dioxide capacity increased almost to normal values. The expiratory quotient fell to, and remained at, distinctly subnormal values. The hydrogen ion concentration of the blood returned almost to normal despite the increasing pressure of carbon dioxide in the expired air.

Special attention is called to the relatively small change in pulmonary ventilation, virtually none, accompanying a relatively large increase in gaseous metabolism. These results agree with those recently described by Eddy (1931) who showed that the effects of methylene blue on pulmonary ventilation vary, depending upon the prevailing conditions. If injected under normal conditions, an increase in pulmonary ventilation

invariably occurs, but if injected during hyperpnea, elicited by intravenous injection of cyanide, pulmonary ventilation may be depressed. The conclusion from such results is that the effects of increased oxidations on pulmonary ventilation vary, depending upon the conditions obtaining when oxidations are changed.

Experiment 2 shows striking changes during administration of cyanide and during recovery. During the first part of cyanide administration the animal reacted vigorously to impaired oxidations; ventilation, mean blood pressure and carbon dioxide elimination increased. Then the condition of the animal suddenly changed. The mean blood pressure fell, pulmonary ventilation decreased, oxygen consumption fell off rapidly, carbon dioxide elimination diminished, lactic acid accumulated at a faster rate and the hydrogen ion concentration of the arterial blood increased. During this stage the decrease in carbon dioxide capacity of the blood and the increase in lactic acid content of the blood ran more parallel than during the early period of administration. At the end of cyanide injection, there was a rapid return of hyperventilation accompanied by an increased consumption of oxygen, an increased elimination of carbon dioxide, a reduction in blood lactic acid, a fall in the expiratory quotient and a reduction in the hydrogen ion concentration of the blood. The temporarily increased ventilation occurring during the period of recovery stands in contrast to the decrease in ventilation occurring at the close of injection in experiments 1, 3 and 4. It is probably due to the fact that an acute depression occurred during the administration of cyanide and that during gas samples 10, 11, 12 and 13 the animal is passing from a condition of depression to one of recovery, permitting the cyanide to evoke a second stimulation.

On the whole, experiments 1, 3 and 4 showed that the greater the ventilation the lower was the carbon dioxide pressure of the expired air. Experiment 2 is an exception to these findings. During gas samples 7 and 8 where ventilation was falling off there was a decrease in the carbon dioxide pressure of the expired air and during the later samples in which ventilation was augmented, with the exception of gas sample 9, the carbon dioxide pressure of the expired air increased. These contrary findings may be related to the failure of the circulation to carry carbon dioxide to the lungs during gas samples 7 and 8 when the blood pressure was falling rapidly and to the improved transport of accumulated carbon dioxide when mean blood pressure and volume flow of blood was again increased during gas samples 9, 10, 11, and 12. The dissipation of energy, aerobic and anaerobic, is analyzed in the preceding paper. See figures 1 and 2 and table 1. When oxidations were impaired by the administration of gaseous mixtures low in oxygen it will be recalled that the total oxygen consumption was slightly increased. In the present experiments, on the


SAMPLES
BLOOD TIME
MMHg
cc/kg/hr
NaCN INJECTION
BLOOD pH
MEAN BLOOD PRESSURE
BLOOD CO2 CAPACITY
BLOOD LACTIC ACID
O2 CONSUMPTION
CO2 ELIMINATION
O2 PARTIAL PRESSURE
CO2 PARTIAL PRESSURE

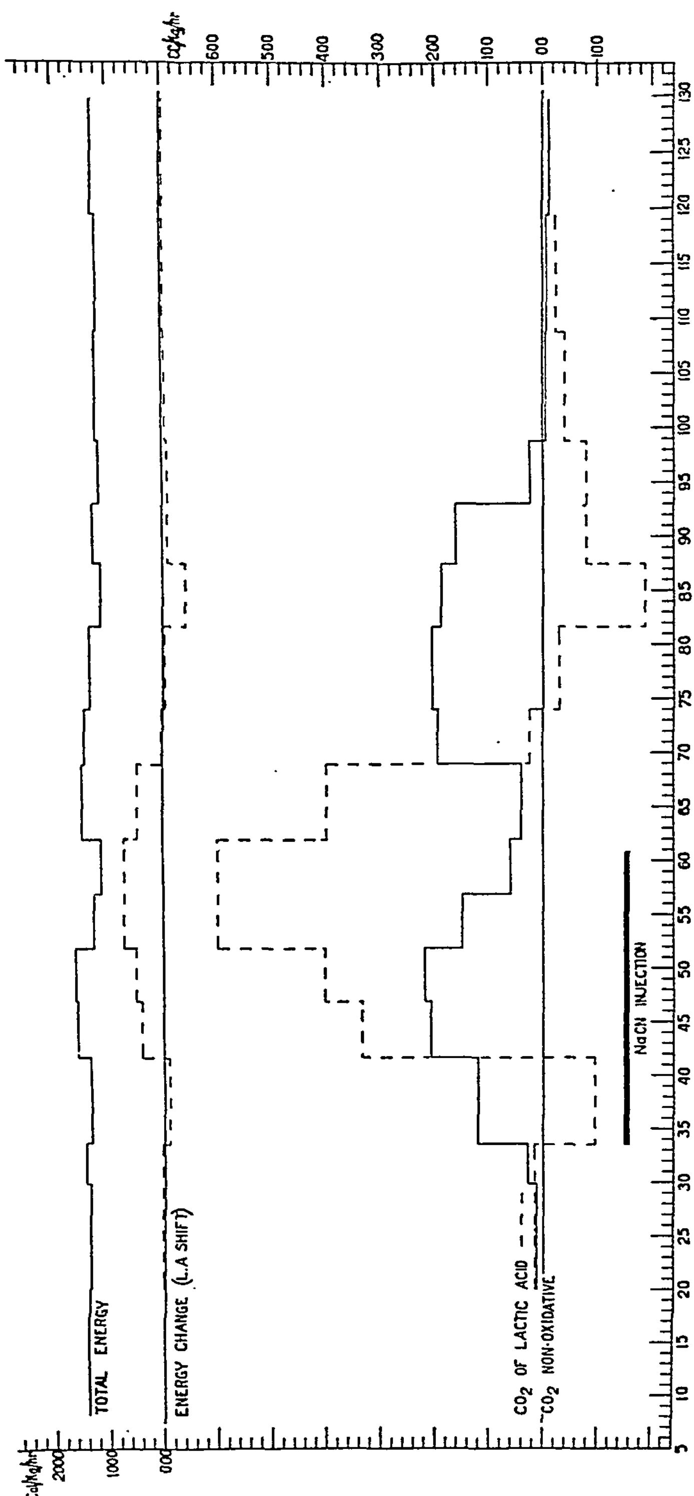


Fig. 2. Experiment 2, August 13, 1930

TABLE 1

Energy relationships

	EXPERIMENT 1			EXPERIMENT 2			EXPERIMENT 3			EXPERIMENT 4		
	NaCN G. S. 4-11	Re-covery G. S. 12-15	NaCN and recovery G. S. 4-15	NaCN G. S. 4-8	Re-covery G. S. 9-17	NaCN and recovery G. S. 4-17	NaCN G. S. 4-11	Re-covery G. S. 12-16	NaCN and recovery G. S. 4-16	NaCN G. S. 4-10	Re-covery G. S. 11-14	NaCN and recovery G. S. 4-14
1. Basal O_2 consumption	358.2	263.4	621.6	135.2	323.0	458.2	260.3	194.7	455.0	213.8	202.4	416.2
2. Observed O_2 consumption	324.1	215.8	539.9	95.3	296.1	391.4	217.1	145.5	362.6	188.7	196.6	385.3
3. Oxygen balance	−34.1	−47.6	−81.7	−39.9	−26.9	−66.8	−43.2	−49.2	−92.4	−25.1	−5.8	−30.9
4. Anaerobic energy	+53.7	−2.3	+51.4	+35.5	+1.0	+36.5	+41.0	−10.8	+30.2	+38.1	−8.0	+30.1
5. (3) + (4)	+19.6	−49.9	−30.3	−4.4	−25.9	−30.3	−2.2	−60.0	−62.2	+13.0	−13.8	−0.8
6. O_2 work (ventilation)	−0.2	−0.1	−0.3	−0.2	−0.2	−0.4	−0.4	−0.2	−0.6	−0.3	−0.1	−0.4
7. O_2 warming air	−6.2	−3.4	−9.6	−4.5	−6.5	−11.0	−7.2	−4.3	−11.5	−5.6	−2.5	−8.1
8. O_2 saturating air	−24.5	−13.7	−38.2	−17.8	−25.9	−43.7	−28.6	−16.9	−45.5	−22.3	−9.7	−32.0
9. O_2 CO_2 liberation	−13.4	−3.8	−17.2	−7.1	−6.2	−13.3	−12.6	−7.0	−19.6	−10.0	+0.3	−9.7
10. (6) + (7) + (8) + (9)	−44.3	−21.0	−65.3	−29.6	−38.8	−68.4	−48.8	−28.4	−77.2	−38.2	−12.0	−50.2
11. (5) + (10)	−24.7	−70.9	−95.6	−34.0	−64.7	−98.7	−51.0	−88.4	−139.4	−25.2	−25.8	−51.0

All values are given in cubic centimeters of oxygen per kilogram.

contrary, oxygen consumption was definitely reduced (see figs. 1 and 2, and items 1 and 2 in column 1 of each experiment in table 1). The oxygen balance is given in line 3. The anaerobic energy liberated in terms of cubic centimeters of oxygen is given in line 4. Line 5 (the sum of the oxygen balance and the anaerobic energy) indicates a deficit of energy below an anticipated basal rate equivalent to 2.2 and 4.4 cc. in experiments 3 and 2. In experiments 4 and 1 an excess above an anticipated basal rate amounting to 13.0 to 19.6 cc. occurred. These results differ from those of the low oxygen experiments in which the energy liberated during anoxemia was never less than the expected basal rate. The dissipation of energy during the recovery period differs still more from that of the lowered alveolar oxygen experiments in which oxidations were always markedly increased over the expected basal rate. In the cyanide experiments, oxidations during the period of recovery were always less than the expected basal rate as seen by a comparison of items 1 and 2 in column 2 of each experiment. Since lactic acid also decreased during this period the sum total of energy dissipated was less than the anticipated basal rate. The sum total of dissipated energy for the combined periods of administration and recovery (see item 5, column 3 of each experiment) was in every instance less than the anticipated basal rate. Adding this deficit to the energy required for the extra pulmonary ventilation, the warming and the saturating of the extra air with water vapor, and the energy of liberation of the extra carbon dioxide from the blood into the alveolar air gives the total computed negative energy balance. In experiments 1, 2, 3 and 4 there was a deficit of energy in oxygen equivalents amounting to 95.6 cc., 98.7 cc., 139.4 cc., and 51.0 cc. per kgm. In the three experiments in which gaseous mixtures low in oxygen were administered the same method of computations gave a positive balance of energy. A comparison of the results of the low oxygen and cyanide experiments would thus seem to indicate that impairment of oxidation by interference with the oxidative mechanism is more harmful than interference with the supply of oxygen. Whether this is due to a greater tissue acidity resulting from impaired transport of carbon dioxide by a relatively highly oxygenated blood, or to some other cause, is difficult to say.

In the preceding experiments the formation of lactic acid and its distribution between blood plasma and the corpuscles, striated muscle and testicles were discussed with the aid of tabulated determinations. In the cyanide experiments determination of the lactic acid content of blood and tissues were also made. They appear in table 2 and the order of concentration is summarized in table 3. In experiment 3 determinations were not made on the content of lactic acid in plasma and in the corpuscle, but only in whole blood, muscle and testicle. In general the highest

concentration obtained in muscle, the lowest in the testicle and the intermediate concentration in plasma, blood and corpuscles during basal metabolic conditions. In three experiments testicular content was lowest and in one the corpuscles had a slightly smaller content.

During the administration of cyanide there was a general increase in lactic acid content for blood and tissues but not an equal increase as is

TABLE 2

Distribution of lactic acid before and after intravenous injection of sodium cyanide

EXPERIMENT		BLOOD LACTIC ACID	PLASMA LACTIC ACID	CORPUSCLE LACTIC ACID	MUSCLE LACTIC ACID	TESTICLE LACTIC ACID
		mgm./100 cc.	*mgm./100 cc.*	*mgm./100 cc.*	*mgm./100 grams*	*mgm./100 grams*
1	Basal	20.7	16.7	22	34	12
	After NaCN	99.1	111	75.8	62	60
	Increase	78.4	94.3	53.8	28	48
2	Basal	31.8	45.1	26.5	38	20
	After NaCN	91	138.2	73.3	91	68
	Increase	59.2	93.1	46.8	53	44
3	Basal	25.5			30	13
	After NaCN	86			75	50
	Increase	60.5			45	37
4	Basal	25.6	37.5	20.8	41	21
	After NaCN	84.4	98.6	61.6	68	57
	Increase	58.8	61.6	40.8	27	36

TABLE 3

Order of lactic acid concentration

EXPERIMENT	BEFORE INTRAVENOUS INJECTION OF SODIUM CYANIDE					AFTER INTRAVENOUS INJECTION OF SODIUM CYANIDE				
1	M	C	B	P	T	P	B	C	M	T
2	P	M	B	C	T	P	B	M	C	T
3	M		B		T		B	M		T
4	M	P	B	T	C	P	B	M	C	T

shown in the lower half of table 3. In all of the experiments in which plasma determinations were made, plasma concentration is now seen to be the highest. Due to the large increase in plasma lactic acid, blood concentration is second in order, muscle concentration has fallen from highest to third highest concentration, corpuscular concentration is fourth highest and testicular concentration remains the lowest. The results are very similar to the experiments on lowered alveolar oxygen. The mecha-

nisms leading to a basal distribution and effecting an altered distribution during impaired oxidations are probably the same and have been discussed in the preceding paper.

SUMMARY AND CONCLUSIONS

In a preceding series of experiments effects of intravenous injection of sodium cyanide were studied during administration of uniform ventilation with room air. In the present series of experiments the effects of injection were studied with pulmonary ventilation under physiological control.

A 0.004 M. sodium cyanide solution was injected 25 to 48 minutes at a rate sufficient to produce marked augmentation of pulmonary ventilation.

The results obtained are compared with those of cyanide injection and uniform ventilation and with those of lowered alveolar oxygen with uniform ventilation and with physiologically controlled ventilation.

In both series of cyanide experiments oxygen consumption was greatly decreased during the period of injection. These results are similar to the reduction of oxygen consumption accompanying the administration of gaseous mixtures low in oxygen during uniform ventilation but differ from the effects of lowered alveolar oxygen when ventilation is under normal control.

Hyper-oxygen consumption, common to recovery after oxygen lack, was missing during recovery from cyanide injection. Oxygen consumption of the recovery period was below the preceding basal rate.

Carbon dioxide elimination was decreased by cyanide injection during uniform artificial ventilation, but increased during physiologically controlled ventilation.

During recovery, with uniform ventilation, carbon dioxide elimination increased towards normal with a tendency to overshoot. With ventilation under physiological control carbon dioxide elimination decreased towards normal with a tendency to undershoot the basal rate.

The reduction in carbon dioxide elimination from cyanide injection during uniform ventilation was attributed to lowered oxidations and to impaired transport of carbon dioxide from the tissues to the lungs resulting from a permanently high oxygenation of the blood. The increased elimination of carbon dioxide during cyanide injection and physiological control of ventilation was attributed to the washing out of preformed carbon dioxide by augmented ventilation.

The increase in carbon dioxide elimination and tendency toward overshooting during recovery from cyanide under uniform ventilation was attributed to the increase in oxidations and improved transport of hyper-accumulated carbon dioxide in the tissues. The reduction in carbon dioxide elimination during recovery with physiologically controlled ventilation was attributed to the reduction in ventilation, to the relatively

desaturated condition of the tissues and blood, and to a more complete reversal of the lactic acid cycle and liberation of free base.

The expiratory quotient during cyanide injection and uniform ventilation was slightly increased, during physiologically controlled ventilation it was markedly increased.

The increase in the expiratory quotient during cyanide injection and uniform ventilation is attributed to the formation of lactic acid.

The relatively small increase in the expiratory quotient compared with that of lowered alveolar oxygen and uniform ventilation is tentatively attributed to an impairment in transport of carbon dioxide associated with a permanently high oxygenation of the blood. This inefficient transport is in contrast to an augmented carrying power, the result of a permanent low oxygenation of blood, obtaining with lowered alveolar oxygen.

The large increase in the expiratory quotient during cyanide injection and physiologically controlled ventilation is attributed to the augmented ventilation.

During recovery from cyanide injection during uniform ventilation, the expiratory quotient diminished, but not to subnormal levels as was the case in lowered alveolar oxygen during uniform ventilation. This relatively high recovery expiratory quotient is tentatively attributed to the combined effects of a greater accumulation of carbon dioxide in the tissues and to a more incomplete recovery of the oxidative mechanism of the cell with consequent failure of removal of lactic acid.

During recovery, under physiologically controlled ventilation, the expiratory quotient fell slightly below normal. This greater reduction in the recovery expiratory quotient, as compared with that of uniform ventilation, is attributed to a lower carbon dioxide content of the tissues at the time of returning oxidations and to a more complete recovery of the tissues permitting a more perfect reversal of the lactic acid cycle with liberation of free base. The relatively small reduction in the expiratory quotient as compared with the lowered alveolar oxygen experiments is again attributed to greater content of carbon dioxide in the tissues and a less perfect recovery of cellular functions.

Blood lactic acid increased during cyanide injection in both series of experiments and decreased during recovery. The recovery decrease was greater in the experiments in which ventilation was physiologically controlled. Since in neither series were the animals suffering from lack of oxygenation of the blood, it is concluded that the better recovery in the experiments with physiologically controlled ventilation may be attributable to the greater elimination of carbon dioxide.

The relative order of concentration of lactic acid before injection of cyanide (muscle, plasma, whole blood, corpuscle, and testicle) was changed

at the end of injection to plasma, whole blood, muscle, corpuscles and testicles. These results are similar to those produced by lowered alveolar oxygen and are tentatively interpreted in the same way.

The carbon dioxide capacity of the blood was decreased during cyanide administration and increased during recovery. The changes were smaller than the corresponding changes in blood lactic acid. A tendency towards a slight and temporary increase in carbon dioxide capacity during the early part of cyanide injection was noted. The results agree in general with those of cyanide injection and uniform ventilation and of lowered alveolar oxygen.

The hydrogen ion concentration of the blood decreased during cyanide injection and increased towards normal during recovery with a tendency towards slight temporary overshooting. With uniform ventilation there was an increased hydrogen ion concentration during injection followed by a tendency towards recovery. Since cyanide injection and uniform ventilation were associated with a decreased alveolar carbon dioxide pressure, the increase in hydrogen ion concentration was attributed to an increased lactic acid content and an increased oxygenation of the blood. Since the periods of cyanide injection during physiologically controlled ventilation were accompanied by an increased lactic acid content and increased oxygenation of the blood, the decreased hydrogen ion concentration is attributed primarily to the increased ventilation.

During the first part of the period of cyanide injection the total energy production (oxidative plus non-oxidative) was greater than the basal energy production. This gave way to a decreased production as oxidations were more markedly impaired. Subnormal dissipation of energy continued during recovery despite the tendency towards improved oxidations. In every case the combined period of administration and recovery showed a negative energy balance as compared with the basal rate. This negative balance coupled with the superbasal requirements for the extra work required to move the air, of warming the extra air, of saturation of the extra air with water vapor, etc., establishes a large deficit in required dissipation of energy.

The fact that a given augmentation of ventilation produced by cyanide administration is associated with a greater deficit of energy than a similar augmentation of ventilation produced by lowered alveolar oxygen suggests why cyanide administration is more harmful.

The intravenous administration of methylene blue during recovery from cyanide injection markedly accelerated the chemical changes usually associated with recovery.

Despite a sudden augmentation in the formation of carbon dioxide and in the partial pressure of carbon dioxide of the expired air there was a decrease in the hydrogen ion concentration of the arterial blood. This

decrease in hydrogen ion concentration was associated with a sharp decrease in blood lactic acid and an increase in the carbon dioxide capacity of the blood and a relative retention of carbon dioxide as indicated by the drop in the respiratory quotient to subnormal levels.

Despite the sudden increase in oxidations, with its consequent increased formation of carbon dioxide, ventilation remained virtually unchanged. This finding agrees with those of Eddy (1931) showing that methylene blue may increase or decrease the magnitude of pulmonary ventilation depending on the rate of oxidations obtaining at the time of injection.

The finding is also in agreement with the observation of Miss Pelecovich (1932) that the relation of pulmonary ventilation to the degree of impairment of oxidation is variable and uncertain.

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THE EFFECT OF FASTING UPON THE ACTIVITY OF SKELETAL MUSCLE[1]

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The purpose of this investigation was to determine the effect of varying periods of fasting upon the activity of the intact skeletal muscle of the rat. The phases of activity studied were the number of artificial stimuli required to produce fatigue and the amount of work accomplished by the muscle during the total period of activity. Studies were made upon the glycogen content of muscle and liver from the exercised and control animals in order to determine whether a relationship existed between the amount of glycogen stores and muscular activity.

PROCEDURE. Adult rats selected from the general laboratory stock were weighed and placed in separate cages and subjected to fasting for periods varying from 24 to 144 hours in duration. Water ad libitum was allowed throughout the fasting period. The animals designated as non-fasting were taken directly from the stock cages which contained at all times an abundant diet of soy beans, corn, dog biscuits, milk and greens. A satisfactory degree of surgical anesthesia was produced by the intraperitoneal injection of 6 mgm. of amytal per 100 grams of body weight. Additional amounts of anesthetic were administered from time to time as needed. The extent of the superficial reflexes was used as a criterion of the depth of anesthesia. Control and experimental animals were subjected to the same depth and duration of anesthesia.

The gastrocnemius muscle was exercised by maximal faradic break shocks under conditions which permitted the utilization of its natural attachments, thus avoiding as far as possible disturbances due to distortion or overstretching. The leg was fixed in a rigid position by a modified femur clamp attached to the tibia. A cord was fastened to the foot by a pin hook which was thrust through the middle phalangeal joints

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of the toes. This cord was attached to a muscle lever weighted with a load equal to one half of the animal's weight. Flexible glass shielded electrodes were attached to the tendon achilles and gastrocnemius muscle. A motor driven device equipped with a cyclometer was employed to deliver supermaximal break induction shocks to the muscle at the rate of 2 to 3 per second. The muscular contractions were recorded on a slow moving extension kymograph. The muscle was considered to be fatigued when there occurred no perceptible movement of the lever. The amount of work done was calculated from the load lifted and the height of the contractions. Corrections were made for the magnification due to leverage.

TABLE 1

A summary of the effects of activity and fasting upon muscle and liver glycogen

(Mgm. per 100 grams of tissue)

	LIVER		MUSCLE	
	Control	Exercised	Control	Exercised
Non-fast	2,185	164	537	125
P.E.M	±585	±23	±61	±16
24 hr. fast	172	67	370	74
P.E.M	±15	±15	±8	±9
48 hr. fast	78	58	375	56
P.E.M	±19	±9	±20	±11
80 hr. fast	254	72	356	90
P.E.M	±29	±9	±7	±12
120 hr. fast	345	46	321	109
P.E.M	±49	±7	±11	±8
144 hr. fast	339	84	355	131
P.E.M	±70	±16	±14	±7

At the conclusion of the experiment the gastrocnemius muscle and liver were quickly excised, weighed and placed in hot 60 per cent NaOH. The glycogen was determined according to the method of Pflüger as modified by Bollman, Mann and Magath (1925). The sugar content of the hydrolyzed glycogen was determined by the method of Shaffer and Hartmann (1920). In order to learn something as to the general condition of the animal, determinations of the lactic acid content of heart's blood were made at the end of some of the experiments.

Results. The mean values for the glycogen content of liver and muscle from the fatigue and control rats are presented in table 1. They represent an average of determinations made on eight or more animals

in each group. The values for the probable error of the mean are presented to indicate something as to the variations in individual experiments. It was found that a fast of 24 hours materially reduced the glycogen content of muscle. Longer periods of fasting did not bring about any further reduction in muscle glycogen. Fasting markedly decreased the glycogen content of the liver. The lowest values were found at the end of a 48 hour period. Apparently liver glycogen diminished rapidly in the early hours of fasting and then increased during the longer periods to values well above the minimal. Exercise caused a great reduction in muscle and liver glycogen. However, demonstrable quanti-

TABLE 2

A summary of the effect of fasting upon body weight and activity of intact gastrocnemius muscle

	NON-FAST	24 HR. FAST	48 HR. FAST	80 HR. FAST	120 HR. FAST	144 HR. FAST
Per cent loss of body weight		6.0	9.5	16.9	20.9	22.5
Stimuli to fatigue in thousands	67	71	67	95	69	116
Probable error of mean	±6	±10	±10	±10	±6	±9
Time in hours to fatigue	6.7	7.3	6.7	11.4	7.9	12.0
Probable error of mean	±0.6	±0.9	±1.0	±1.6	±0.6	±1.1
Total work* per gram of muscle	2.8	2.7	2.8	3.2	2.0	3.1
Probable error of mean	±0.3	±0.4	±0.4	±0.5	±0.2	±0.6
Work* per gram of muscle during 1st 1000 contractions	0.26	0.24	0.28	0.27	0.18	0.22
Probable error of mean	±0.02	±0.02	±0.01	±0.02	±0.02	±0.03
Work* per kgm. of original body weight	13.4	11.8	12.0	15.3	9.5	15.1
Probable error of mean	±1.9	±1.4	±1.6	±2.6	±0.9	±2.7

* Work in kilogram-meters.

ties of glycogen were present in the muscle and liver at a time when the muscle was considered to be in a state of complete fatigue. No relationship appeared to exist between the time required to fatigue or the amount of work performed and the quantity of muscle and liver glycogen present before and after fatigue. Although considerable variation existed in the individual experiments, it was apparent that fasting did not affect the amount of work done by a muscle or the number of stimuli required to produce fatigue (table 2). The amount of work done by the muscle has been expressed in terms of the number of kilogram meters per gram of gastrocnemius muscle, kilogram meters per kilogram of body weight

before the beginning of the fast, and the amount of work accomplished during the first 1000 contractions. Muscles from the 120 hour fasting group exhibited the least susceptibility to fatigue as judged by the time required and the amount of work performed during activity. The average values for the lactic acid content of heart's blood from the fatigued animals was approximately twice that found in the anesthetized controls.

Discussion. The work on the effects of fasting upon the activity of man and animals is entirely too voluminous to be discussed here. Readers are referred to Morgulis (1923) for an excellent account of many such experiments. In general, the work of previous investigators has shown that fasting and under-nutrition are accompanied by diminished ability to do work and an increased susceptibility to fatigue. A few instances are recorded in which an individual's strength was apparently increased for a short time during a fast and in which isolated muscles from fasting animals showed a diminished rate of fatigue. Many of these observations were made under experimental conditions which did not permit one to draw conclusions as to whether the effects of fasting upon activity were related to alterations in the stimulus or the ability of the muscle to respond. In our work we have attempted to control the former factor by applying directly to intact muscle electrical stimuli of strengths greater than that required to produce maximal contractions. It is believed that the preservation of the natural muscular attachments permits one to approach more nearly the true physiological conditions of the natural activity of muscle. It was our experience that this arrangement permitted a muscle to do much more work than when the cut tendon method was employed. We believe the lessened activity found when the latter method was employed to be due to an unavoidable interference with the circulation in the muscle due to its distortion or overstretching. The values found for the lactic acid content of heart's blood at the end of the experiments indicate that the failure of the muscle to exhibit further contractions was not due to a collapse of the systemic circulation and respiration. The total amount of work done by kilogram of original body weight is regarded as the most reliable of the various criteria of activity employed (table 2). The total number of stimuli and the total time required to produce fatigue are regarded as less significant because of the difficulty of determining the exact time at which the muscle no longer exhibited perceptible shortening. In some experiments the muscles exhibited a very slight degree of shortening for several hours. In other experiments the duration of this period of slight activity was much shorter.

It is apparent from the results of these experiments that the skeletal muscle from animals that had been fasted for periods as long as 144 hours was able to perform as much work as muscle from control non-fasted animals. The mean values observed in the experiments on the 144 hour

group are suggestive of an increased efficiency during fasting. However, when one considers the range of variation in the individual experiments and the fact that the activity of the 80 hour fasted group was slightly less than that of the controls, it is apparent that this suggestion cannot be regarded as significant. No studies were made on animals fasted for periods longer than 144 hours. We wished to avoid, as far as possible, the complications that might arise from the use of anesthesia for long periods in the terminal stages of fasting. The amount of work performed and the rate of fatigue were found to be independent of the amounts of glycogen contained in the liver and muscle at the beginning and at the end of the experiments. Muscles from animals with glycogen poor livers exhibited as much activity as muscles from animals having a normal glycogen content. At the conclusion of the experiment demonstrable quantities of glycogen were found to be present in the liver and exercised muscle of all animals. There appeared to be no relationship between the amount of work done by a muscle and the amount of glycogen depletion in the liver and muscle. Our findings are in harmony with the observations of Witting, Markowitz and Mann (1930) who found the survival time of perfused glycogen poor hearts to be as long as that of hearts with normal amounts of glycogen.

SUMMARY

A study has been made of the effects of fasting upon the activity of the artificially stimulated intact gastrocnemius muscle of the anesthetized rat. The phases of activity studied were the number of stimuli required to produce fatigue and the amount of work done by the muscle. The results indicate that fasting for periods of 24 to 144 hours did not affect either the rate of fatigue or the total amount of work done by the artificially stimulated intact muscle. Studies were made upon the glycogen content of liver and muscle from control and exercised animals. In all experiments, fatigue occurred before an exhaustion of liver and muscle glycogen. The amount of work performed by the muscle and the rate of fatigue were found to be independent of the amount of glycogen present in liver and muscle at the beginning and at the end of the experiments.

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THE EFFECT OF DISLOCATION OF CEREBROSPINAL FLUID UPON ITS PRESSURE

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In the interpretation of the changes in the pressure of the cerebrospinal fluid, caused by tilting a four-footed animal from the horizontal to the vertical positions, stress was necessarily laid upon the possible dislocation of the fluid from the uppermost portions of the nervous system into the dependent (Weed, 1929b). The measurements of the pressure of the cerebrospinal fluid were made, in this series of experiments, by means of the customary open-end manometer of small bore, slightly over 1 mm. in diameter. The pressures obtained by this method have always been taken to be a fairly accurate record of those existing within the subarachnoid space; but there are of course certain hypothetical objections to the use of a system of such small bore, particularly when the fluid in the manometer is normal saline solution or cerebrospinal fluid. In the 1 mm. system, filled with these fluids, the theoretically upward pull of the fluid, due to capillary forces, has been assumed to be of no significance, as the continued pulsations of the fluid constantly alter the surface tension of the meniscus and thereby eliminate any capillary pull.

In the customary group of experimental conditions necessitating measurement of the pressure of the cerebrospinal fluid, the open-end manometer of small bore has, because of its simplicity, proven eminently satisfactory. It has always been realized that such an open-end manometer, either as a straight apparatus or as a U-shaped tube for measuring negative pressures, introduces the element of atmospheric pressure more or less directly upon the central nervous system. For under the conditions of measurement of the fluid-pressure, the meniscus of fluid in the manometer must be taken as indicating the level of atmospheric pressure. While in most experiments upon the cerebrospinal fluid the readings in the open-end manometer will serve as an accurate guide to pressure-changes within the subarachnoid space, they cannot be assumed to be as exact as those obtained in a system which permits measurement without dislocation of fluid. The most satisfactory method of determining fluid-pressures without dislocation is that of the so-called "bubble manometer" in which a bubble of air is confined within a glass tube of small bore. The pressures

are read by the height of the column of fluid needed to maintain the bubble in an arbitrarily established position, constituting zero (or atmospheric) pressure when the distal end of the system beyond the bubble is filled with fluid and open to the atmosphere. Any increase or decrease in this pressure, on connection to the animal, is represented by a dislocation of the imprisoned air-bubble; adjustment of the reservoir restores the bubble to the previous position, so that determination of the pressure imparted by the reservoir of fluid gives the record of the pressures within the animal without dislocation of fluid.

While it has proven true that pressures of the cerebrospinal fluid may be obtained with a fair degree of accuracy in manometers of small bore and of open end, certain types of experiments have aroused question as to the employment of this method in those cases where great differences in the pressures recorded involve the displacement of relatively large amounts of fluid. Our own attention was acutely called to this problem by the great pressure-alterations obtained in the cerebrospinal fluid on abrupt tiltings of the animal from the horizontal to the vertical head-down and tail-down positions (Weed, 1929a, b; Weed and Flexner, 1932). In these experiments pressure-changes of 200 mm. were not uncommon. As such changes involved a considerable dislocation of fluid, even with a 1 mm. manometer, it was thought that a comparison of the findings with different manometers in living animals and in artificial systems might be of interest. The results brought out a definite and important relationship between the dislocation of cerebrospinal fluid and its pressure.

METHODS OF INVESTIGATION. A series of small dogs of fairly uniform size (weight, 6–7 kgm.) was employed for the experiments. The animals were all given ether and attached to a tilting board. The customary puncture into the subarachnoid space through the occipito-atlantoid ligament was made on each animal and the needle connected to manometers of different bores. In the usual experiment the manometer, initially attached, was a modification of the bubble manometer, in which a single bubble of air was imprisoned in a long glass tube of approximately 1 mm. bore. The initial readings with the bubble manometer were followed by connection of open-end manometers of 1 mm., 2 mm., 4 mm., 6 mm., 8 mm., 10 mm., and in some experiments 16 mm., 26 mm., and 45 mm. bore.

In each instance, with the attachment of the different manometers, the fluid pressure was restored to approximately the pre-existing level for the animal in horizontal position. The animals were then tilted to the vertical positions, head-down and tail-down, and readings of the pressure of the cerebrospinal fluid taken. At the conclusion of the experiments the animals were killed.

In addition to the observations made on living animals a series of similar experiments was carried out on a system of glass tubes and tam-

bours, which simulated conditions in the living animal. This artificial system consisted of a straight glass tube, 400 mm. in length (corresponding to the length of the spinal column in the dogs used). Attached to this tube was a cross-bar for connection of the manometer and an extension of a 70 mm. tube, representing the head-end of the dog beyond the needle. The system was so arranged that tambours of different diameter, covered with rubber membranes, could be attached at either end. The artificial apparatus was filled with fluid after connection of manometers, and positive pressures of the desired degree obtained from a fluid reservoir. The elastic membrane of the tambour permitted the maintenance of these positive pressures by distending the membrane. The whole glass tube-tambour system was attached to the tilting table so that readings could be taken in a horizontal position and subsequently in positions corresponding to the vertical head-down and tail-down positions of the experimental animal. With possible dislocation of fluid into the open-end manometer or into the dependent tambour, a series of observations of the pressure-changes was made with the various manometers used on the experimental animal.

EXPERIMENTAL FINDINGS. With the series of manometers graded both by bore and by capacity, the two series of observations—one on the living animal and one on the artificial system,—were carried out. The results of these two types of experiments are given below.

Experimental animals. In the anesthetized dog, the bubble manometer gave readings of the pressure of the cerebrospinal fluid in the horizontal position of the same magnitude as with an open-end, small-bore manometer. This pressure was usually in the neighborhood of 125 mm. of normal saline solution, but due to differences in age and some individual variation, these horizontal pressures varied somewhat from this mean. The pressure-alterations in the cerebrospinal fluid on tilting, as determined by the closed manometer, were slightly greater than with the ordinary 1 mm. manometer, but these differences were so slight as to call attention to the general accuracy of all readings obtained by the open-end 1 mm. manometer, as customarily employed in determinations of the pressure of the cerebrospinal fluid. With the larger manometers, all of the pressure-alterations varied in accordance with the bore of the manometer, the larger manometers giving much smaller differences in the pressure-changes on tilting from the horizontal to the vertical positions.

These generalizations are best illustrated by the record of a single animal, in which a series of observations was made with manometers of different bore and with the bubble manometer attached at the beginning and the end of the experiment. Chart 1 shows such a record, carried out continuously on an etherized dog. The pressures have been arbitrarily brought back to a common base line, about 200 mm. positive pressure,

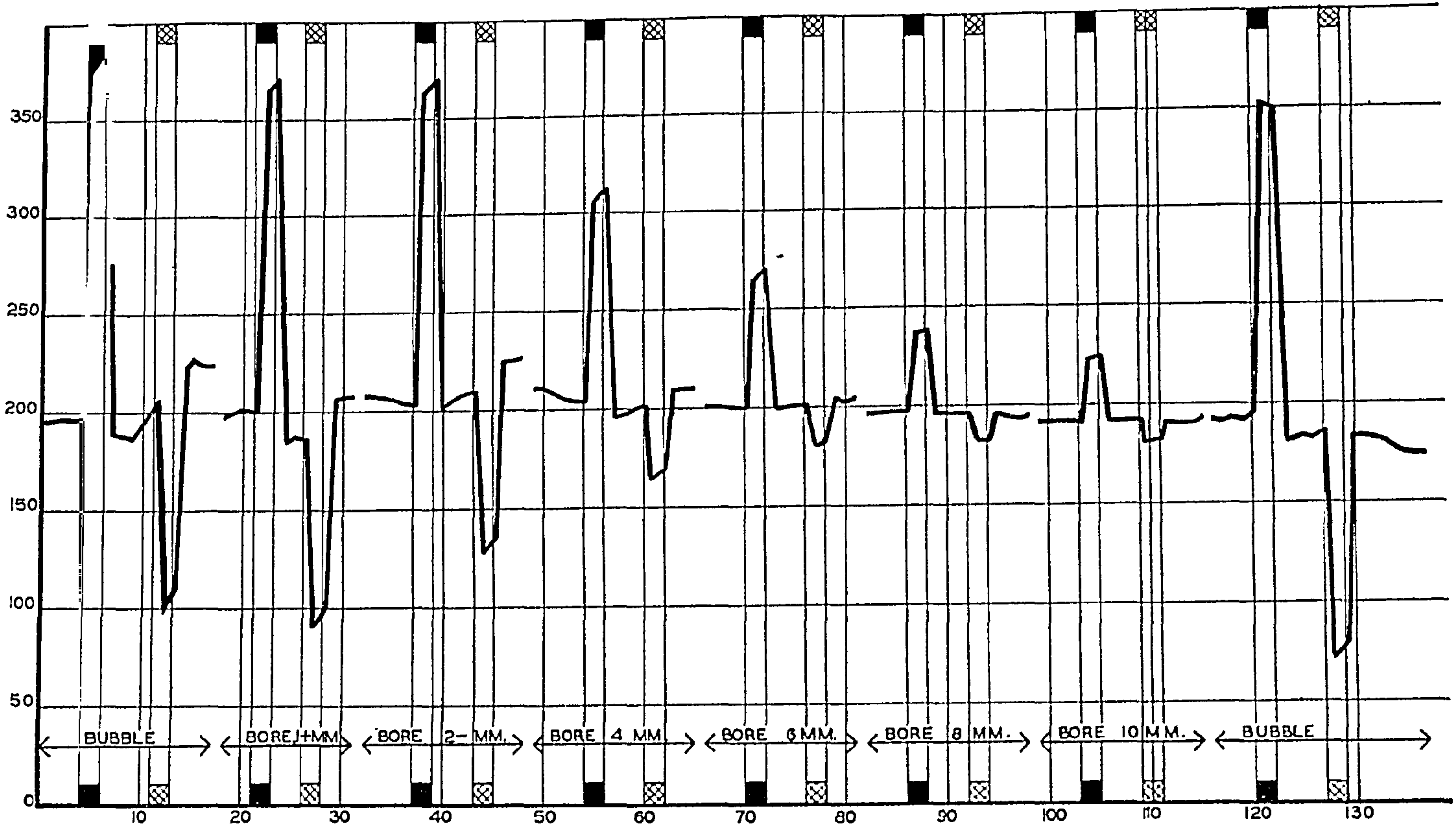

Chart 1 (dog, expt. A-100). The ordinates represent millimeters of normal saline solution; the abscissae, time in minutes. The chart records the pressure of the cerebrospinal fluid from an occipital manometer. The animal was in the horizontal position except during intervals marked by the solid blocks when it was tilted to the vertical head-down position, and during intervals marked by the cross-hatchings when it was tilted to the vertical tail-down position. As indicated on the chart, the pressure of the cerebrospinal fluid was recorded by bubble manometer and by open-end manometers of various bores (from 1 mm. to 10 mm.).

so that ease of comparison of the magnitude of the alterations could be attained. In the first period of this experiment the bubble manometer was used, permitting observations of pressure without dislocation of fluid. Here shift from the horizontal to the vertical head-down position resulted in an increase of pressure of 177 mm. in the cerebrospinal fluid, while the tilt from the horizontal to the tail-down position gave a pressure-reduction of 112 mm. With the substitution of the manometer of approximately 1 mm. bore the vertical head-down tilt gave a pressure-increase of 167 mm., while the vertical tail-down shift showed a decrease of 94 mm. As progressively larger manometers were used, the pressure-changes in the cerebrospinal fluid on tilting to the two vertical positions became smaller and smaller, as shown in chart 1. After this series with open-end manometers was completed, the bubble manometer was again attached; and on tilting from the horizontal to the vertical head-down position a pressure-increase of 157 mm. was noted, while on tilting from the horizontal to the vertical tail-down position a decrease of 111 mm. was recorded.

In the particular experiment recorded in chart 1, no use was made of manometers larger than 10 mm. bore. When these larger manometers were used, the results were quite in accord with the series illustrated in chart 1. Thus in experiment A-95, the pressure of the cerebrospinal fluid when recorded by the bubble manometer, rose 135 mm. on tilting to the vertical head-down position, and fell 79 mm. on the opposite vertical tilt. With a manometer of 26 mm. bore, the increase in pressure of the cerebrospinal fluid, on the vertical head-down tilt, was noted to be 4 mm., while the decrease in pressure on tilting from the horizontal to the vertical tail-down position was 2 mm. The difficulty of accurate reading, with such small changes in the height of the fluid level and with the large meniscus of fluid, was even more strikingly brought out in the attachment of a manometer of 45 mm. caliber. Because the determination of the level in a manometer of such great bore could not be accurately made, the readings were not continued, although they showed results which were quite in keeping with the decreasing pressure-alterations noted in the larger and larger manometers.

Consideration of the results obtained with the open-end manometers of different bore in comparison with the pressure-alterations noted in the bubble manometer, led us to speculate regarding the relation of dislocation of fluid to the pressure recorded. It was realized that as the bore of the manometer increased a greater and greater dislocation of fluid from the central nervous system into the manometer was necessary to achieve any given pressure-alteration on the vertical head-down tilts, while on the vertical tail-down shifts the dislocation of fluid was from the manometer into the central nervous system. It was obvious that the base-line of

pressure-alteration was necessarily that attained by the bubble manometer, for in this case no dislocation of fluid into or from the manometer system could be assumed, and such alterations in pressure as were measured by the bubble manometer represented true pressure-alterations without dislocation of fluid into or from any external system.

In a series of experiments the manometers were all calibrated as to the volume of fluid dislocated in the pressure-alterations of the magnitude obtained. The determination of the capacity of the manometers was made in two ways and the results checked against each other. By the first of these methods actual weight of mercury contained in the manometer in the pressure-change noted was determined; and in the second method, a theoretical computation of the weight of the fluid made. The two determinations checked on some of the manometers within a fairly close range, indicating that these manometers were of fairly accurate bore throughout; others of the manometers showed far greater inequalities in caliber.

It was found that these determinations of the volume of displaced fluid had a fairly constant relationship to the pressure-changes obtained in any one animal. This relationship was expressed in a fraction or quotient designated as $\frac{dV}{dP}$, where dV represents the difference in volume between the experiment with no displacement (bubble manometer) and the actual cubic centimeter change in any of the open-end manometers, while dP represents the difference in centimeters of the pressure-change on tilting, between that recorded by the bubble manometer and those by any of the open-end manometers. Calculating this fraction, $\frac{dV}{dP}$, from the results of the tiltings to the vertical head-down and tail-down positions a fairly constant value was obtained in most of the experiments. This is shown in table 1, which represents the data from two typical experiments, both carried out on dogs of the more or less standard size and weight used in these observations. In the whole series, the fraction $\frac{dV}{dP}$ was found to have an average value of 0.17. Calculations of the value of the fraction $\frac{dV}{dP}$ may be also made by using the pressure-changes from any two of the open-end manometers with their volume-differences; and by this method of determination similar values are obtained.

Consideration of these findings led to the generalization that the coverings of the central nervous system and cerebrospinal fluid may be likened to a container of unknown volume with elastic elements of an unknown coefficient of elasticity. This encasement approximates a rigid container because of the low elasticity of the walls and the character of the enclosing

bony sheath. According to such a conception it becomes possible to consider the application of the physical laws of an elastic system. The general expression for any coefficient of elasticity is the quotient obtained by dividing the stress by the strain. In the coefficient of volume-elasticity E, the stress is the change in pressure dP and the strain is the change in volume dV divided by the original volume V. Or, E = coefficient of elasticity $= dP/\frac{dV}{V} = \frac{dP}{dV}V$.

In the central nervous system, the volume-elasticity of the membranes leads to a dislocation of fluid, amounting to the volume dV, which is

TABLE 1

Table showing the derivation of the fraction $\frac{dV}{dP}$ from two dogs (A-95 and 99), giving relationship between the pressure of the cerebrospinal fluid and the volume of fluid dislocated

MANOMETER BORE	HEAD-DOWN				TAIL-DOWN				EXP. NO.
	Pressure-change	Difference in pressure-change	Volume Dis-placed	$\frac{dV}{dP}$	Pressure-change	Difference in pressure-change	Volume Dis-placed	$\frac{dV}{dP}$	
	cm.	*cm.*	*cc.*		*cm.*	*cm.*	*cc.*		
Bubble	14.8	0	0		3.8	0	0		A-99
1 mm.	13.3	1.5	0.231	0.154	3.4	0.4	0.059	0.148	
4 mm.	8.1	6.7	0.879	0.131	2.3	1.5	0.228	0.152	
8 mm.	3.3	11.5	1.68	0.146	0.9	2.9	0.458	0.158	
26 mm.	0.4	14.4	2.58	0.179	0.1	3.7	0.645	0.174	
Bubble	13.5	0	0		7.9	0	0		A-95
1 mm.	12.0	1.5	0.192	0.128	7.3	0.6	0.116	0.193	
4 mm.	7.1	6.4	0.770	0.120	5.5	2.4	0.480	0.200	
8 mm.	2.8	10.7	1.43	0.134	1.5	6.4	0.900	0.141	
26 mm.	0.4	13.1	2.58	0.197	0.2	7.7	1.29	0.167	

related to a change in pressure dP. The ratio $\frac{dV}{dP}$, derived above from our experiments, may be considered to be equal to $\frac{V}{E}$ where V is the original volume of the fluid-system (intradural contents) and where E is the coefficient of elasticity of the physiological system which here is a complex function involving vascular tone as well as membrane elasticity. In any one animal, the normal volume V does not seem to vary appreciably as any theoretical or actual dislocation of cerebrospinal fluid may be entirely or partially compensated by a reciprocal readjustment within the vascular channels. Assuming then that the volume V in any one

animal has a normal value within the limits of this reciprocal vascular readjustment, one would expect the fraction $\frac{dV}{dP}$ to remain constant as long as the animal remained in good condition with normal responses. In most of our experiments this was found to hold true. There were some animals however in which the fraction was different for the head-down and tail-down tilt. As V was the same, this difference indicated an abnormality in the physiological response to positional change. The most extreme variations in any one animal were between the limits of 0.048 (tail-down tilt) and 0.223 (head-down tilt). In this particular animal the pressure-changes on head-down tiltings gave a fair degree of

TABLE 2

Table showing derivation of the fraction $\frac{dV}{dP}$ in a dog (A-100) on vertical head-down and tail-down tiltings with manometers of various calibers

The pressure-change set down for the bubble manometer, 17.9 cm., represents the average of the first two tiltings (177 mm. and 181 mm.) on head-down tiltings while the corresponding figure for tail-down tiltings 11.2 cm., is the first of the two readings (112 mm. and 111 mm.).

MANOMETER BORE	HEAD-DOWN				TAIL-DOWN			
	Pressure-change	Difference in pressure-change	Volume Displaced	$\frac{dV}{dP}$	Pressure-change	Difference in pressure-change	Volume Displaced	$\frac{dV}{dP}$
	cm.	*cm.*	*cc.*		*cm.*	*cm.*	*cc.*	
Bubble	17.9	0	0		11.2	0	0	
1 mm.	16.7	1.2	0.267	0.223	9.4	1.8	0.150	0.083
2 mm.	16.0	1.9	0.290	0.152	8.3	2.9	0.150	0.051
4 mm.	10.2	7.7	1.012	0.131	3.7	7.5	0.367	0.049
6 mm.	6.7	11.2	1.77	0.158	1.7	9.5	0.46	0.048
8 mm.	3.8	14.1	1.934	0.137	1.1	10.1	0.558	0.056
10 mm.	3.2	14.7	2.28	0.155	0.9	10.3	0.638	0.062

constancy to the fraction as did also the pressure-changes on tail-down tiltings (table 2), indicating a marked difference in the elasticity of the system in these two vertical positions. Minor alterations in elasticity occurred in many experiments, resulting in slightly variable reactions to tiltings. Thus in this same animal (A-100), the pressure-increases obtained with the bubble-manometer on tilting from the horizontal to the vertical head-down position were 177, 181, and after an interval, 157, 161, 174, 168 mm., on observations throughout the period of experimentation; the pressure-decreases on tail-down tiltings were 112 and 111 mm. Under these circumstances it seemed necessary to determine the magnitude of the pressure-alterations with the bubble-manometer as a series,

taking the average of the alterations rather than a single observation for the establishment of the base-line of reference. This inconstancy of results in positional pressure-alteration is graphically shown in chart 4 of a previous report (Weed, 1929b), in which repeated tilting to the tail-down position was practiced, and may be interpreted as due to minor variations in the physiological elasticity of the system.

Provided no marked alteration in elasticity takes place throughout an experiment, resulting in a constant value for the fraction $\frac{dV}{dP}$, it should be possible to calculate the change in volume of the intradural contents from a recorded change in pressure, and in this way to determine the amount of fluid abstracted from the nervous system by the intravenous injection of a strongly hypertonic solution (vid. Weed and McKibben, 1919a, b; Weed and Hughson, 1921a, b, c, et al.). In other words, the amount of brain shrinkage and loss of cerebrospinal fluid, uncompensated by reciprocal dilatation of the vascular bed (vid. Weil, Zeiss and Cleveland, 1931), might be computed by multiplying the pressure-change in the cerebrospinal fluid by the factor $\frac{dV}{dP}$, thus determining the unknown volume-change within the membranes of the central nervous system.

To this end a series of experiments on etherized dogs of the same size was undertaken. Pressures of the cerebrospinal fluid were determined in the horizontal position throughout by attachment of a U-manometer (1 mm. bore, capable of recording negative pressures), to a needle inserted into the subarachnoid space through the occipito-atlantoid ligament. The animal (B-1), after an initial period of control, was given intravenously 20 cc. of 30 per cent solution of NaCl and subsequently an additional intravenous injection of 10 cc. of the same solution. The pressure of the cerebrospinal fluid dropped rapidly, and 25 minutes after the completion of the intravenous injection a negative pressure, hovering about minus 150 mm., was attained. The total alteration in the pressure of the cerebrospinal fluid was at this time 23.0 cm. (from plus 80 mm. to minus 150 mm.). If this pressure-alteration be multiplied by the quotient (0.17), the product 3.91 should indicate in cubic centimeters the actual diminution in the intradural contents, effected by the hypertonic solution. To test experimentally this computation, a very small puncture hole was made through the calvarium and cranial dura, and a bland mineral oil in the amount of 4.4 cc. was injected into the cerebral subarachnoid space from a finely calibrated syringe. The pressure of the cerebrospinal fluid rose to plus 90 mm.—10 mm. above the initial reading in this animal. Multiplying this pressure-difference (24.0 cm.—from minus 150 mm. to plus 90 mm.) by the factor 0.17, gives a product of 4.08 cc., to be contrasted with the actual injection of 4.4 cc. The difference between the

actual and computed values here is decreased when correction of 0.37 cc. is made for the fluid displaced into the manometer during the rise from minus 150 mm. to plus 90 mm.

Subsequently in this animal, the fluid of the central nervous system was removed by drainage from the needle, and the injected oil in the cerebral subdural and subarachnoid spaces abstracted as far as possible. After such removals, the pressure in the subarachnoid space was again determined in the occipital manometer, and oil in different amount was again injected through the small cranial opening. The injections were repeated several times in the animal, with different amounts of the oil, in order to test out the correspondence over a wide range. In all cases, the correction for fluid displaced into the manometer was applied to the

TABLE 3

Table compiled from results obtained on dog (expt. B-1)

This animal, whose cerebrospinal fluid pressure was measured by open-end manometer of 1 mm. bore, was given 30 cc. 30 per cent NaCl intravenously. When the nervous system was dehydrated, bland oil was injected intradurally in various amounts and the pressure-increase in the cerebrospinal fluid determined.

C.S.F. PRESSURE-CHANGE	$\frac{dV}{dP}$	COMPUTED $\frac{dV}{dP} \times$ PRESSURE-CHANGE	CORRECTED COMPUTATION	OIL ACTUALLY INJECTED UNDER DURA
cm.		*cc.*	*cc.*	*cc.*
11.5	0.17	1.79	1.96	1.8
18.3	0.17	3.11	3.41	3.0
15.0	0.17	2.55	2.79	2.6
3.1	0.17	0.52	0.57	0.6
3.9	0.17	0.66	0.72	0.8
12.1	0.17	2.06	2.26	2.2
24.0	0.17	4.08	4.45	4.4

calculations; the correction was relatively small and did not disturb the correspondence between the theoretical and actual determinations. The whole series of results is given in table 3.

In other cases, the exact agreement of results noted in this experiment was not obtained. This can probably be attributed to slight alterations in the elasticity of the system in the course of the experiment and consequent alterations in the value of the fraction $\frac{dV}{dP}$.

This fraction would only remain equal to 0.17 throughout an entire experiment in ideal cases and would vary in value from dog to dog owing to differences in volume V. In the great majority of cases, however, a number of observations gave results of striking identity between the actual amount of fluid injected and the computed amount as determined

by multiplication of the pressure-change by the fraction $\frac{dV}{dP}$ with its average value of 0.17.

In still other experiments, the introduction of fluid into the space about the dehydrated nervous system was made from the manometer and in these cases normal saline solution was substituted for the mineral oil. Here again some inconstancy of results was obtained; but on the whole the determination of the volume-change effected by means of the hypertonic solutions was amazingly accurate as judged by the correspondence between the amounts of foreign solution actually injected with the calculated value as derived by multiplying the observed pressure-increase by the factor 0.17.

Tambour-tube system. By use of the glass-tube system with attached tambours, many series of observations were undertaken, employing manometers of different bores for comparison with the findings as obtained by the bubble-manometer. The elasticity of the system, represented in the covering of the tambour, was a determining factor in the magnitude of the reactions, both in the system which permitted no external dislocation of fluid and in that permitting dislocation of fluid into the open-end manometer. Not only were experiments made with a single tambour attached at either end, corresponding to the tail or head position of the living animal, but other observations with a tambour at each end were carried out. The results were quite similar; and, like the experiments recorded previously (Weed and Flexner, 1932), no essential difference was observed on tiltings from the horizontal to either of the vertical positions.

In such a system of rigid tubes and elastic membrane attached in the tail position, the difference on tilting between the readings with the bubble-manometer and those with the open-end manometer of 1 mm. bore was far greater than in the living animal. As one employed manometers of larger and larger bore, the pressure-changes diminished quite as they did in the animal series. Furthermore, under the conditions of experimentation, the membrane was distended in the horizontal position in order that a positive pressure might be obtained. In those experiments in which almost no dislocation of fluid occurred, this distention of the membrane had no influence on the character of the shift,—that is, on whether the shift was to the vertical head-down or tail-down position. When however manometers of larger bore were employed, with a greater dislocation of fluid, the head-down shifts almost always gave a larger increase in pressure than the decrease in pressure obtained on shifts from the horizontal to the vertical tail-down position. This finding was interpreted as being due to the fact that in such shifts to the tail-down position fluid was dislocated from the manometer into the glass tube-tambour

system causing further distention of the membrane at the caudal end of the system. This further distention taxed the elasticity of the membrane more than the partial collapse of the distended membrane when fluid was dislocated into the manometer in the contrariwise shift. The difference in response of the rubber membrane offers the explanation for this difference in pressure-alterations.

As computations had been made on the constancy of the factor $\frac{dV}{dP}$ in experimental animals on such tiltings from the horizontal to the vertical positions, similar calculations were made from the pressure-alterations derived from the rigid tube-tambour system when connected to the various

TABLE 4

Record of pressure-readings obtained from glass tube-manometer system for determination of $\frac{dV}{dP}$

Glass tube of 400 mm. length; single tambour of 35 mm. diameter attached in "tail" position. Tambour covered with glove rubber of medium thickness.

MANOMETER BORE	HEAD-DOWN				TAIL-DOWN			
	Pressure-change	Difference in pressure-change	Volume Displaced	$\frac{dV}{dP}$	Pressure-change	Difference in pressure-change	Volume Displaced	$\frac{dV}{dP}$
	cm.	*cm.*	*cc.*		*cm.*	*cm.*	*cc.*	
Bubble	36.5	0	0		36.3	0	0	
1 mm.	28.1	8.4	0.488	0.058	27.8	8.5	0.483	0.057
2 mm.	27.0	9.5	0.572	0.060	26.6	9.7	0.564	0.058
4 mm.	14.4	22.1	1.428	0.064	11.6	24.7	1.151	0.047
6 mm.	7.0	29.5	1.848	0.062	5.8	30.5	1.531	0.050
8 mm.	3.9	32.6	1.985	0.061	3.2	33.1	1.628	0.049
10 mm.	2.8	33.7	1.994	0.059	2.3	34.0	1.637	0.048

types of manometers. The results of a single experiment of this type are given in table 4 for the tiltings from the horizontal to the two vertical positions, with the bubble manometer and with the open-end manometers of various bores.

The remarkable constancy of the factor $\frac{dV}{dP}$ in this physical system, simulating the experimental animal, is of great interest when examined in comparison with the findings given in table 1. For the tiltings from the horizontal to the vertical head-down position, in which some collapse of the partially distended rubber membrane may be assumed, the factor is quite constant. This is true also of the tiltings to the vertical tail-down position with the 1 mm. and 2 mm. manometers. With the larger

manometers, particularly when the dislocation of fluid exceeded 1 cc., the factor becomes slightly smaller, but is still well within the limits of the experimental error.

It was found that when the elasticity of the system was altered by using different rubber membranes or by using tambours of different diameters, factors $\frac{dV}{dP}$ of entirely different magnitude were derived, yet these factors also were remarkably constant for each experiment.

Discussion. The results reported in the foregoing pages seem quite striking in showing a relation between the dislocation of the cerebrospinal fluid and its pressure. One observation of practical importance is the fact that, with the usual manometer of 1 mm. bore, pressure-changes in the cerebrospinal fluid, even those of great magnitude, are quite similar to the readings obtained with the bubble-manometer where no dislocation of fluid occurs. These results are of interest in demonstrating that the use of this open-end manometer is satisfactory for observations of the changes occurring in the cerebrospinal fluid in most types of experimentation.

Aside from these more or less practical considerations, the series of experiments on the living animal demonstrated a definite and fairly constant relationship between the dislocation of cerebrospinal fluid and its pressure, expressed in the ratio $\frac{dV}{dP}$. This ratio, computed from the change in volume of the dislocated fluid, divided by the change in pressure, is theoretically a function of the total volume V and of the elasticity of the system. In any one animal or in those of uniform size, where V does not vary appreciably, the fraction $\frac{dV}{dP}$ should remain constant provided no alteration in elasticity occurs. This was found to hold true. Hence, having calculated the value of this fraction for one normal animal, it should be possible to apply it to other animals of approximately the same size and weight, with systems of normal elasticity, for calculation of the decrease in volume of the intradural contents occurring as a result of dehydration. This was done successfully and demonstrates the interesting and important fact that, within the same species, the elasticity factor is constant enough in normal animals to permit calculations of volume-changes from observed pressure-changes. Consequently, observations made simultaneously on pressure and volume changes should permit a theoretical determination of total volume.

In the course of these experiments it was found that a similar fraction had been calculated by Ayala (1923) in studies of the cerebrospinal fluid in clinical cases. In his first paper he computed what he called a Rachidealquotient, $\frac{Q \cdot F}{I}$, where Q is the amount of cerebrospinal fluid removed,

F the final pressure, and I the initial pressure. Ayala could not assign any definite mathematical value to his quotient, $\frac{Q \cdot F}{I}$; but as it varied directly with the fluid removed and with the final pressure, as well as inversely with the initial pressure, Ayala considered that it had clinical value in giving an idea of the relationship between the quantity of cerebrospinal fluid and its pressure. In a long series of cases the quotient was found by Ayala to range from 1.66 to 20.69, depending upon the character of the pathological lesion within the nervous system.

While the variation in Ayala's Rachidealquotient seems extreme in pathological conditions, the index has some diagnostic importance in the hands of various competent observers (cf. Ayer, 1926; Balduzzi, 1924). Later Ayala (1925) modified the quotient, deriving it by dividing the difference between the initial and final pressure by the quantity of cerebrospinal fluid removed by lumbar puncture. This is the reciprocal of the fraction $\frac{dV}{dP}$ used in this paper. Ayala stated correctly that the value of his quotient is an expression of the volume of the cerebrospinal fluid and the elasticity of the system. Assuming that physiological elasticity does not vary greatly, he considered the quotient as an index of volume and found, from his clinical results, an indication of smaller volumes in cases of brain tumor. As he dealt only with pathological cases the value of his quotient was not constant and the variations he found may have been due to changes in volume, as he supposed, or to changes in both volume and elasticity.

In our experiments, holding the volume as invariable as possible by using animals of the same size, we have demonstrated that the value of the fraction is remarkably constant, indicating but little variation in the elasticity of the physiological system in normal animals of the same species. Once the value of the coefficient of elasticity is determined, the formula can be applied to a calculation of volume-change with change in pressure, or to a calculation of total volume from simultaneous volume and pressure-changes.

The very similar results obtained with a system of rigid tubes and rubber membranes show that the physiological mechanisms involved can be considered as elastic systems. They apparently concern the elasticity not only of the anatomical coverings of the nervous system (the cerebrospinal fluid channels) but also of the blood vascular system. The elasticity of the dura against outward distention seems to be very small indeed. Particularly is this true in the intact animal, for in the cranial region the dura is closely applied to the bony skull and therefore constitutes a membrane of great rigidity—surely of far greater rigidity than the spinal dura, suspended in the epidural space with its areolar tissue and thin walled

veins. The fact that there is in most animals a constant relationship between the fluid dislocated and the resultant pressure of the cerebrospinal fluid indicates a considerable elasticity which permits a certain dislocation of fluid even in the intact animal (Weed, 1929a, b; Weed and Flexner, 1932). This dislocation of fluid may be due to the elasticity (collapse inward) of the spinal dura on the vertical head-down tiltings, but it also seems related fundamentally to the possible compression or dilatation of the blood vessels. Because of the high pressure existing in the arteries of the central nervous system in comparison to that of the veins, it would seem important to emphasize the elasticity of the venous system as a factor in the establishment of the relation of dislocation of the fluid to its pressure rather than the elasticity of the arterial channels.

In those animals in which the fraction $\frac{dV}{dP}$ is constant, it seems safe to assume an elasticity of the system almost as perfect as that of the rubber membranes in the tambours. Were the elasticity of an imperfect character, varying with different loads and with different amounts of dislocation of fluid, no constant fraction could possibly be obtained.

We have then in the central nervous system a mechanism which relates the dislocation of cerebrospinal fluid to the resultant pressure. The constancy of this relationship is remarkable in those animals where the intradural volume is the same: it indicates that the whole central nervous system may be looked upon as enclosed within elastic membranes whose function is modified by the rigid character of the bony encasement.

SUMMARY

Pressures of the cerebrospinal fluid were measured by bubble manometer and by open-end manometers of various bores in dogs of similar weight and size. Pressures recorded in manometers of 1 mm. bore did not vary greatly from those obtained with the bubble manometer. With larger open-end manometers, the pressure-alterations in the cerebrospinal fluid on tilting from the horizontal to the vertical positions, decreased in proportion to the amount of fluid dislocated in the manometer.

In animals of the same size there was a constant relationship between volume of fluid dislocated and pressure-change. By means of this relationship, expressed in the fraction $\frac{dV}{dP}$, accurate determinations were made of the volume-diminution in brain and cerebrospinal fluid, effected by intravenous hypertonic solutions. The value of the fraction $\frac{dV}{dP}$ is a function of the volume of the system and of its physiological elasticity.

The pressure-changes, on tilting from horizontal to vertical positions, were duplicated by an artificial system of glass tubes and tambours

covered with rubber membranes. From this system a similar constant, expressing the relationship between fluid-dislocation and pressure-change was obtained, the magnitude of the fraction depending on the elasticity and capacity of the membranes.

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HETEROTOPIC BONE FORMATION IN THYROPARATHYROIDECTOMIZED DOGS

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We have been attempting by a variety of methods to study the relationship of the parathyroid glands to calcification and ossification. One method that suggested itself as being free from some of the objections to experiments on skeletal growth or repair of fractures in which the questions of osteogenesis from preëxisting bone, periosteum or cartilage may complicate the interpretation, was that of ectopic or heterotopic bone formation. As Phemister (1923) has stated, there exists a tendency to ossification in the organs of the urinary tract, under certain experimental and pathological conditions, which is greater than that in any other system of organs. Two particular types of experimental ossification that have been developed which illustrate this tendency on the part of the urinary tract have been studied. Neuhof (1917) reported the formation of bone in fascial transplants which were used to repair a defect in the wall of the urinary bladder of dogs. This was a consistent finding, occurring in all of eighteen dogs that survived the operative procedure for a long enough period. He reported calcification occurring as early as six days, and ossification occurring as early as seventeen days in some animals, but the height of ossification occurring between six and eight weeks. This has been confirmed by others, notably Phemister (1923), who reported bone formation in every instance even though attempts were made to keep the urine alkaline by diet and administration of alkalis. Huggins (1930, 1931) has confirmed and extended these observations and has shown that bone will form in a fascial implant in the urinary bladder even though the ureters are transplanted so that no urine enters the bladder. In addition Huggins has shown that when an excised portion of the bladder wall or bladder mucosa is stitched so that it overlies the rectus fascia, a cyst will form in the course of twenty days or more, and that in a portion of the wall of this cyst, bone will invariably develop if infection does not occur.

We have performed a number of experiments similar to those of Neuhof and Huggins in two series of thyroparathyroidectomized dogs. A circular patch of the bladder wall about 2 cm. in diameter was excised from the dome of the urinary bladder. This defect was then filled by stitching in a

circular patch of fascia lata with chromic catgut. The excised portion of the bladder wall was then stitched to the anterior sheath of the rectus abdominis muscle. Both procedures were carried out in some animals, while in others only one was performed. For convenience in reporting the results in tabular form we have designated the patch of fascia lata transplanted into the wall of the urinary bladder as a "Neuhof patch," and the patch of the urinary bladder wall stitched to the rectus fascia as a "Huggins patch." In one series of thyroparathyroidectomized dogs intensive calcium therapy was instituted. Calcium lactate was given by mouth, with supplementary calcium gluconate[1] intramuscularly or intravenously. An effort was made to keep the animals free of tetany and to keep the level of the blood calcium as near the normal as possible. The results in this series are indicated in the following table:

TABLE 1

Heterotopic bone formation in thyroparathyroidectomized dogs. Intensive calcium therapy

N = "Neuhof Patch." H = "Huggins Patch."

DOG NUMBER	DAYS	RESULT
1*	63	Bone present—H
2	49	Bone present—N
3	56	Bone present—N and H
4	62	Bone present—N
5	63	Bone present—N
6*	66	Bone present—N and H
7	59	Bone present—H

* Dogs 1 and 6 did not show tetany during test periods of calcium withdrawal. Figure 1 illustrates the bone formation typical of this group.

In the second series of thyroparathyroidectomized dogs, the effort was made to keep the animals alive with minimal calcium administration, with the object of maintaining low blood calcium levels for as much of the time as possible. As is indicated in the following table a number of animals succumbed from tetany before a sufficiently long period elapsed to draw conclusions. It was possible, however, to get several to survive for the period ordinarily required for bone formation.

Discussion. It is not pertinent to this paper to discuss the local factors which condition these types of heterotopic osteogenesis. Readers are referred to Huggins (1931) for a consideration of this aspect of the problem. It is quite clear, however, from the experiments in the first series of dogs that heterotopic bone formation can and does occur in the

[1] We are indebted to Mr. E. D. Marti of the Sandoz Chemical Works for the supply of calcium gluconate used in these experiments.

Fig. 1

Fig. 2

Fig. 1. Portion of urinary bladder wall of dog showing fascial implant ("Neuhof patch"). Thyroparathyroidectomy with intensive calcium administration and resulting bone formation.

Fig. 2. Portion of urinary bladder wall of dog showing fascial implant ("Neuhof patch"). Thyroparathyroidectomy with minimal calcium administration and no bone formation.

TABLE 2

Heterotopic bone formation in thyroparathyroidectomized dogs. Minimal calcium therapy

N = "Neuhof Patch." H = "Huggins Patch."

DOG NUMBER	DAYS	RESULT
1*	60 and 210	No bone—N
2	19	No bone—N
3	8	No bone—N and H
4	3	No bone—H
5	9	No bone—H
6	6	No bone—N and H
7	25	No bone—N
8	4	No bone—N
9	4	No bone—N
10	5	No bone—N
11	6	No bone—N
12	10	No bone—H
13	5	No bone—H
14	2	No bone—H
15	22	No bone—N
16	2	No bone—N and H
17	64	Bone present—N and H
18	56	No bone—N

* Dog 1. Exploratory laparotomy at 60 days. Sacrificed at 210 days. Figure 2 illustrates a fascial implant into the urinary bladder in which no bone was formed.

dog in the absence of the parathyroid glands if ample calcium is given to maintain the animals free of tetany. Two of the dogs in this group probably did not have a complete parathyroid deficiency as they did not show tetany during a test period when calcium was withheld. The remaining five were apparently completely deficient with respect to parathyroid tissue as demonstrated by repeated attacks of tetany during such test periods. The amount of bone found in these thyroparathyroidectomized dogs seemed in most instances to be practically as great as in similar experiments on normal animals.

From the experiments in the second series there is some indication that this heterotopic bone formation is less likely to occur in thyroparathyroidectomized dogs maintained with minimal calcium therapy. Three animals (nos. 1, 17, and 18) survived a long enough period for bone formation to regularly occur in normal animals, but bone developed in only one. Three additional animals (nos. 2, 7 and 15) survived a long enough period for bone formation to occur frequently in normal animals, but no bone developed. With respect to one of the dogs in this series (no. 1) that did not develop bone in a "Neuhof patch," it can be definitely stated that the blood calcium level was below normal at all times. In this instance the thyroparathyroidectomy had been performed seven months before the operation on the urinary bladder. Calcium administration had been discontinued for a month and was not required to prevent tetany during any of the time that the experiment continued. The blood calcium levels varied between 5.1 and 7.2 mgm. per 100 cc. except on one occasion when a value of 9.1 was observed. In the other animals subnormal blood calcium levels probably occurred during most of the experimental period as tetany was frequent and the minimal amount of calcium administered that would alleviate it.

CONCLUSIONS

1. Heterotopic bone formation can occur in thyroparathyroidectomized dogs receiving adequate calcium therapy in *a*, fascial transplants in the wall of the urinary bladder, and in *b*, the cysts formed by stitching the urinary bladder mucosa to the rectus fascia.
2. There is definite indication that such heterotopic bone formation is less likely to occur in thyroparathyroidectomized dogs receiving minimal calcium therapy.

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THE SPECIFICITY OF PANCREATIC LIPASE: ITS APPEARANCE IN THE BLOOD AFTER PANCREATIC INJURY

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Since the work of Hanriot (1897), Doyon and Morel (1902), and Arthus (1902) it has been known that the serum contains normally no enzyme capable of hydrolyzing the true fats or oils, differing in this respect from pancreatic secretion which is very active on the triglycerides of the higher fatty acids. These same workers recognized that an esterase capable of splitting ethyl butyrate was present in the blood. At the present time, however, it is a common practice to use ethyl butyrate, tributyrin, or triglycerides of the higher fatty acids indifferently as substrates for an enzyme which is referred to as lipase. Enzymes are chiefly distinguished by the substrates on which they act, and Oppenheimer (1925) states that since serum esterase is active on such triglycerides of the lower fatty acids as tributyrin the difference between this esterase and the lipase which hydrolyzes true fats may be questioned.

If the lipase of the pancreas is a specific enzyme and essentially different from the serum esterase one might expect that a lipase attacking true fats would appear in the blood following pancreatic injury and that the concentration of such a lipase in the serum would not parallel that of the normal serum esterase. An increase in serum diastase is known to occur following pancreatic injury; the literature has been reviewed recently by Elman, Arneson, and Graham (1929). Several workers (Hess, 1912; Hiruma, 1923; Diena, 1919) have attempted to demonstrate an increase in blood lipase after pancreatic duct ligation. All of them used the simple esters as substrates, and their results are not in agreement for reasons which will appear. Hewlett (1904) found an increase in esterase (ethyl butyrate) in the urine after pancreatic injury and in one case where olive oil was used as a substrate a lipase was demonstrated.

With these facts in mind it was considered of considerable importance to determine whether "pancreatic" lipase (olive oil splitting lipase) appears in the blood after pancreatic injury.

Method. The outflow of secretion from all or part of the pancreas was obstructed in 10 dogs. In 6 of these both pancreatic ducts were ligated, in

one animal the tail of the pancreas was doubly ligated and cut between ligatures, and in the remaining three the head and tail were both ligated and the body of the pancreas removed, leaving head and tail free in the mesentery. Determinations of the lipolytic activity of the serum were made prior to operation and at frequent intervals postoperatively, using ethyl butyrate and olive oil as substrates. As further controls more than 50 determinations on 30 normal animals under similar conditions of diet and subjected to various surgical procedures (gastrectomy, biliary fistula, section of splanchnics, ureteral ligation, London cannula on portal vein) were made.

A modification of the Loevenhart method for esterase was employed; to 1 cc. of serum were added 3 cc. distilled water, 1 cc. absolute ethyl butyrate, and 0.5 cc. N/3 potassium phosphate buffer adjusted to pH 7.0 (3 cc. N/3 potassium dihydrogen phosphate plus 10 cc. N/3 potassium hydrogen phosphate). This mixture was shaken thoroughly and allowed to stand in the incubator at 40°C. for 24 hours. It was then titrated with N/20 sodium hydroxide, using 3 drops 1 per cent phenolphthalein as an indicator and bringing the solution to the faintest permanent pink.

Some difficulty was experienced in finding a preparation of olive oil which would undergo no change on standing, which would be a sufficiently fine emulsion to present the maximum surface to the action of the lipase and would not settle out to any marked extent on standing, and which could be readily duplicated when more material was required. A 50 per cent emulsion of olive oil in water using 5 per cent of acacia as the emulsifying agent was found to be suitable. The olive oil was freed from all but faint traces of free fatty acid before use. A preservative was necessary and it was found that sodium benzoate was highly satisfactory, not inhibiting the lipolytic action nor interfering with the stability of the emulsion; the concentration of sodium benzoate used was 0.2 per cent. The emulsion was run through an homogenizer 10 to 12 times, which resulted in a very fine and uniform size of oil droplets. The emulsion as described was kindly prepared for us by Dr. D. L. Tabern of the Abbot Laboratories. In testing the action of lipases on olive oil 2 cc. of the above emulsion were added to 1 cc. of serum, and 3 cc. of distilled water and 0.5 cc. of normal phosphate buffer pH 7.0 added as in the ethyl butyrate method. Incubation and titration were the same as for the ethyl butyrate method except that 3.0 cc. of 95 per cent alcohol were added to each tube before titrating.

A series of substrates was employed to determine whether further information on the specificity of the lipases or esterases of serum, liver, and pancreas could be obtained. In all cases the method described for ethyl butyrate was used, substituting 1 cc. of the ester to be studied for the 1 cc. of absolute ethyl butyrate. Extracts of liver and pancreas were made as described below. The esters studied were ethyl, amyl and benzyl

acetate, methyl and iso-amyl salicylate, tributyrin, triacetin, iso-amyl caproate, benzyl succinate, benzyl butyrate, ethylene glycol diacetate, iso-amyl benzoate, ethyl mandelate, ethyl butyl and octyl esters of olive oil, benzyl oleate, and ethyl and butyl stearate.

Since Rona and his co-workers believe that tributyrin is an adequate substrate for pancreatic lipase a separate series of experiments was designed to test this assumption. Determinations of serum lipolytic activity were made in 7 dogs, 3 with ligation of both pancreatic ducts and 4 with ligation of the common bile duct.[1] The hydrolysis of tributyrin was followed both by the titration method as above and by stalagmometry; parallel observations on the hydrolysis of ethyl butyrate and olive oil were carried out.

Other determinations were made to study the distribution of olive oil and ethyl butyrate splitting enzymes in various tissues. Brain, lung, muscle, kidney, spleen, liver, intestinal mucosa, and pancreas were utilized. To 20 grams of the ground tissue 80 cc. of glycerol were added, the mixture shaken and allowed to stand 24 hours. For use the extract was diluted with an equal volume of water and 1 cc. used in place of serum in the methods described above. A similar study, much more extensive in scope, has been reported by Porter (1916). Unfortunately she did not adjust the pH by the use of buffer, and the adequacy of her reported control determinations may be questioned. She found enzymes capable of splitting true oils and fats to be present in varying amounts in various animal species, such a lipase being present in at least one species for each tissue examined. The highest values were obtained from pancreas, liver, and thymus. She did not study dog tissues.

In all the above experiments control tubes employing serum or tissue extract inactivated by heat were used. After a large number of controls had been run on serum, it was found that control tubes employing the same lot of substrate were constant within 0.1 cc. N/20 NaOH; since this was within the limits of error of the determinations, one control was allowed to serve for a number of sera.

Results. Data on 5 of the dogs with pancreatic damage are presented in table 1; results on the other 5 (all with simple duct ligation) are similar. The important fact demonstrated is that in all these animals an olive oil splitting lipase appeared in the blood, the amounts found being surprisingly large. It is interesting to note that in 3 of the 5 animals the maximum value was reached within 24 hours after the operation. Dog 3 in which the maximum value was not reached until the 5th day, refused food post-operatively and died on the 7th day of pneumonia and fat necrosis. Gould and Carlson (1911) following diastase after obstruction to the outflow of

[1] We have found that hepatic injury causes an olive oil splitting lipase to appear in the serum. These observations will subsequently be reported in detail.

pancreatic secretion noted that a secondary rise frequently occurred after the diastase had fallen to normal; the same phenomenon occurred in dog 4 on the 13th day, the lipase rising abruptly almost to its first high level after a gradual postoperative fall. The ethyl butyrate esterase may fall concomitantly with the rise of olive oil lipase, may rise simultaneously, or may show almost no change. Esterase changes, however, are always relatively slight. In our series of 30 control animals, 2 have shown spon-

TABLE 1

Blood esterases in dogs following pancreatic injury

Results expressed in cc. N/20 NaOH. Values for olive oil lipase in columns OO, for ethyl butyrate lipase in columns EB.

DAYS	DUCTS LIGATED		TAIL LIGATED		BODY OF PANCREAS EXCISED					
	Dog 1		Dog 2		Dog 3		Dog 4		Dog 5	
	OO	EB	OO	EB	OO	EB	OO	EB	OO	EB
Control	0.0	2.1	0.0	1.4	0.0	2.3	0.0	1.8	0.0	2.0
1	6.1	1.7	1.0	1.8	0.6	2.0	7.4	1.8	5.0	1.9
2	6.0	0.9	1.2	1.3	0.9	1.8	5.0	1.7		
3	6.6	1.0	0.6	1.6	1.2	1.8	1.8	1.6	5.0	1.7
4			0.2	1.5	1.6	1.8	1.3	1.7		
5	2.0	1.4	0.4	1.3	4.8		1.1	1.7		
6	1.7	2.1	0.1	1.5	1.9	1.4	1.7	1.5		
7	1.1	2.4			Died		1.4			
8	0.4	1.8							2.3	1.5
9	0.0	3.7					2.9	1.8		
10	0.3	3.8								
12	0.1	2.0					0.8	2.1	0.5	2.3
13							6.0	1.9		
14	0.1	2.7								
15							5.6	3.0		
17	0.0	1.2					5.3	6.3	0.4	
20	0.0	1.8					3.8	3.4		
25							3.4	1.8		
29							1.8	3.2		
34							1.8	3.2		
39							3.0	3.7		
42							0.9			

taneously a slight amount of "pancreatic" lipase in the blood; in more than 50 determinations on 28 other animals, including many subjected to various surgical procedures, we have never observed any olive oil lipase in the blood except after liver injury

Our study of 21 various esters showed that in general liver tissue is more active on the simpler esters than are pancreas or serum; the esters of olive oil, oleic acid, and stearic acid were split only very slightly by any of the three; while olive oil emulsion is acted on very strongly by pancreas,

moderately by liver, and not at all by serum. The salicylic acid esters were split only by liver, which Loevenhart (1906) has shown is due to the presence of bile salts. It must be borne in mind that the menstruum in which the enzyme acts may exert a very profound modifying influence in an experiment such as this.

Data on 2 of the seven dogs in which parallel determinations of activity on olive oil, ethyl butyrate, and tributyrin (titration and stalagmometric methods) were made are given in table 2; these results are typical of the group. It is our impression that the stalagmometric method is no more accurate than titration for ethyl butyrate determinations and it is certainly much more laborious.

TABLE 2

Comparative determinations of the activity of serum before and after ligation of the pancreatic ducts on olive oil, ethyl butyrate, and tributyrin (titration and stalagmometric methods)

Results of titration on olive oil, ethyl butyrate, and tributyrin given as cc. N/20 NaOH in 24 hours; on tributyrin by stalagmometry as drops in 1 hour.

DAYS	DOG P19				DOG P20			
	Olive oil	Ethyl butyrate	Tributyrin		Olive oil	Ethyl butyrate	Tributyrin	
			Titration method	Stalagmometric method			Titration method	Stalagmometric method
Control 1	0.1	1.3	2.0	13	0.0	2.0	3.0	18
Control 2	0.0	1.5	2.4	14	0.0	1.8	3.3	21
Postop. 1	3.4	1.3	2.6	15	4.4	2.1	3.6	24
2	4.4	2.0	3.5	18	5.1	2.0	3.7	22
3	4.6	2.2	3.7	22	5.2	1.8	3.6	23
4	1.8	1.6	2.8	19	5.6	2.1	3.4	24
12					0.0	4.4	5.0	29
14					0.1	3.4	4.5	26
16					0.1	4.3	4.8	28

Discussion. We believe that the appearance of an olive oil splitting lipase in the blood after pancreatic obstruction without a definite parallel change in the serum esterase is the best evidence thus far presented for the specificity of the former enzyme. The fact that we have observed increase, decrease, or no change in the esterase under these conditions explains the discordant results of previous investigators who have used simple esters for the study of pancreatic lipase in the blood. Willstatter and Memmen (1924) and more recently Gyotoku (1930) have shown that the action of quinine and atoxyl on the lipolytic enzymes is much modified by accompanying impurities; this fact must be carefully considered in a comparative study of different sources of enzyme. Since under the ex-

perimental conditions which we have used pancreatic lipase appears in and then disappears from the same medium (serum) in which esterase is present without definite alteration of esterase activity, further weight is added to the evidence for specificity. Conditions are certainly more comparable when enzyme concentrations are independently varied in such a relatively constant medium as blood serum than when the sources of the enzymes are extracts of various tissues.

In view of the distinction which may be made between the two types of lipolytic activity, it seems advisable to reserve the term "lipase" for the enzyme active on triglycerides of the higher fatty acids and "esterase" for the enzyme or enzymes which hydrolyze the simpler esters. Hydrolysis of olive oil is accomplished in significant amounts only by pancreas, intestinal mucosa, liver, and spleen, with a trace of activity in the kidney. All tissues studied showed activity toward ethyl butyrate. We are now

TABLE 3

Distribution of olive oil splitting lipase and ethyl butyrate splitting lipase in the tissues

TISSUE	OLIVE OIL	ETHYL BUTYRATE
	cc. N/20 NaOH	*cc. N/20 NaOH*
Pancreas	10.0	1.4
Pancreatic juice	11.5	3.0
Liver	2.4	14.6
Serum	0.0	2.4
Kidney	0.4	6.0
Spleen	2.1	1.7
Lung	0.2	4.1
Intestinal mucosa	6.0	4.0
Brain	0.2	0.3
Striated muscle	0.1	0.8

attempting to determine whether the pancreas is the sole site of lipase formation.

The possibility that several enzymes are concerned in the hydrolysis of the simple esters is suggested by such observations as those of Falk (1924) who found that various tissue extracts hydrolyze different esters in varying amounts. Our results, however, show no significant variation between the hydrolysis of ethyl butyrate and tributyrin by serum following pancreatic obstruction. The parallel rates of hydrolysis of these two esters, on the contrary, suggest that a single enzyme is responsible. Rona and Pavlovic (1922, 1924) using the stalagmometric method with tributyrin as the substrate find that "serum lipase" is inactivated by both quinine and atoxyl, while atoxyl inactivates "liver lipase" and quinine inactivates "pancreatic lipase." These findings have been applied to the diagnosis of diseases of the liver and pancreas on the theory that the specific

lipases appear in the blood, but with highly conflicting results (Segenschmid, 1927; Lowenberg and Kwilecki, 1926; Kwilecki, 1926; Kobryner, 1927; and Grassberger, 1928). The so-called "pancreatic lipase" has been found in serum in pernicious anemia by Simon (1925) and in secondary anemia by Lasch (1928). In a careful experimental study Kromeke (1923) was unable to find any change in the quinine fast lipase following various types of liver injury. The unpredictable behavior of esterase under various conditions may explain the conflicting results of those who have used the stalagomometric method. It is evident from our data that tributyrin hydrolysis is not an index of the presence of true pancreatic lipase. In our hands the method which depends on changes in surface tension (stalagmometry) has no superiority either in accuracy or ease of determination over titration. The division into atoxyl resistant and quinine resistant fractions is based on the properties of organ extracts, and may easily be due to differences in the menstruum.

It is possible that the appearance of lipase in the blood after pancreatic obstruction may be due to the presence under those conditions of an activating agent; we think it unlikely that this is actually the case.

CONCLUSIONS

1. Following obstruction of the pancreas in dogs an olive oil splitting lipase appeared in the blood in large amounts; there was no significant uniform change in esterase as measured by hydrolysis of ethyl butyrate or tributyrin.
2. This is interpreted as evidence of the specificity of lipase; it is suggested that this term "lipase" be reserved for the enzyme (or enzymes) capable of splitting true fats and oils, and that the term esterase be used for the enzyme (or enzymes) acting upon the simple esters.
3. Lipase (olive oil splitting) was found in significant amounts only in pancreas, intestinal mucosa, liver, and spleen. All tissues studied contained an esterase.
4. A study of the activity of pancreatic and liver extracts and blood serum on 21 different esters gave no evidence of specificity other than that indicated by the absence of activity toward olive oil in certain tissues and its presence in others.

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METABOLISM IN YUCATAN: A STUDY OF THE MAYA INDIAN

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In the Nutrition Laboratory's comprehensive program for studying the metabolism of various races, one of the most salient observations has been that of the relatively high basal metabolism of male Maya in Yucatan. The earliest investigator to discover this high metabolism was Williams (1), who found that the basal metabolism of 32 male Maya was, on the average, 5.2 per cent above the standards for white men of the same age, weight, and height, living in the northern part of the United States. The other outstanding feature of Williams' study was that he noted in several instances strikingly low pulse rates. Three years after Williams made his observations, the Harvard Expedition in charge of Shattuck (2) took up the study again, with the findings of Williams in mind, and the measurements were concentrated upon those individuals whom Williams had observed to have a pronouncedly high metabolism and a low pulse rate. Although the second study was carried out only with considerable difficulty and with a yield of protocols by no means satisfactory to any of the workers, an analysis of the returns showed that, in general, the findings of the first investigation were completely confirmed, that is, that the metabolism of male Maya on the average is 5 or 6 per cent above the standards for American white men of European descent and that there are occasional evidences of a pronouncedly low pulse rate. It was pointed out, however, that in those few instances where measurements were made on more than one day, there was a tendency for the metabolism to be somewhat lower after the first day. It was felt that possibly we had to deal with a group of individuals who, although appearing outwardly calm and completely relaxed, were considerably disturbed by the experimental technique, simple though it was, and it was thought that as the subject became more familiar with the technique his metabolism might tend to approach more nearly the true basal level.

In the winter of 1931[1] the problem was again attacked with the idea of making further metabolism measurements, if possible, on those men who

[1] At that time the Department of Genetics of the Carnegie Institution of Washington began an anthropometric study of the Maya Indian.

had on the previous occasions shown a high metabolism; 2, of noting any low pulse rates, and 3, most important of all, of studying the problem raised by Shattuck and Benedict as to whether observations continued on a second and a third day would show a tendency for a lower metabolism. Anthropometric measurements were also made on the subjects whose metabolism was measured.

In the present study all the field observations were made by Steggerda, who was well acquainted with the difficulties that might be encountered. The place where the observations were carried out was the same (Chichen Itzá) as in the two earlier studies. Our measurements were made in February, March, and April, those by Shattuck in January, February, and March, and those by Williams in March, April, and July. Thanks to the close coöperation of Marty Dzib, a native assistant, sympathetic accord was established with the various subjects. The success of these measurements was also due in no small measure to the most helpful coöperation of Mrs. Morris Steggerda. Precautions were taken to guard against any surreptitious eating by the subjects, and we are certain that we were dealing with the post-absorptive metabolism in all cases. The apparatus with which the metabolism measurements were made was the same as that used in the former studies, namely, the field respiration apparatus (3). The instrument was thoroughly tested before its use, and our technique was reviewed at the Nutrition Laboratory in the fall of 1930, before the start of the expedition, in order to insure perfect manipulation of the apparatus. Since the importance was recognized of securing the full coöperation of the subject, particularly as regards physical and mental repose, every concession was made to insure his tranquillity. As a result, it was possible to study the basal metabolism of 30 male Maya, and in every instance three periods were obtained on each of three consecutive days.

Physical characteristics of the subjects. The anthropometric measurements that pertain to the interpretation of basal metabolism observations, namely, the measurements of body weight, height, and sitting height, are given in abstract in the first part of table 1. To note whether our subjects were especially over or under weight, the Pirquet index of state of nutrition (4), that is, the pelidisi $\left(\frac{\sqrt[3]{10 \times \text{weight (gm.)}}}{\text{sitting height (cm.)}}\right)$ has likewise been computed and recorded in the table. The general configuration of male Maya has been considered *in extenso* by Williams and Benedict (1). The physical characteristics of the groups of Maya studied on the first, second, and third expeditions, respectively, were on the average as follows:

Age, 24, 22, and 27 years; body weight (without clothes) 53.7, 52.7, and 54.4 kgm.; height 158, 159, and 156 cm.; sitting height (measured) 82.3, 83.5, and 83.5 cm.; pelidisi 99, 97, and 98.

This comparison indicates that the physical characteristics of the three groups of men were much the same and that the Maya as a whole are short

TABLE 1

Basal metabolism of male Maya in Yucatan (1931 expedition)*

SUBJECT	AGE	WEIGHT (WITHOUT CLOTHES)	HEIGHT	SITTING HEIGHT	PELIDISI	PULSE RATE 1st day	PULSE RATE 2d day	PULSE RATE 3d day	O_2 PER MINUTE 1st day	O_2 PER MINUTE 2d day	O_2 PER MINUTE 3d day	HEAT PRODUCED PER SQ. M. PER 24 HRS.	DEVIATION (AVERAGE) FROM HARRIS-BENEDICT PREDICTION
	years	*kgm.*	*cm.*	*cm.*					*cc.*	*cc.*	*cc.*	*cal.*	*per cent*
M. Dzib**	21	48.1	155	81.3	97	53	54	53	196	191	196	938	−0.9
F. Echemaria	18	43.3	148	78.4	97	53	53	53	173	175	180	917	−4.5
E. May	26	57.7	159	83.5	99	57	53	56	207	222	209	929	−0.2
T. Canul	16	54.8	155	80.1	102	59	57	56	233	229	228	1,047	+7.5
V. Dzib	36	53.1	156	85.9	94	54	54	52	219	216	224	1,011	+14.4
R. Mis	22	52.7	153	81.7	98	66	60	59	209	231	213	1,015	+7.4
E. Puc	27	51.8	149	77.7	103	49	48	49	223	203	210	1,015	+9.7
S. Ceme	21	57.7	157	85.3	98	53	50	60	241	236	251	1,067	+12.1
J. Ceme	35	56.6	158	86.5	95	58	57	56	220	217	219	970	+8.7
D. Balam	24	54.6	153	82.0	99	55	52	60	233	219	245	1,069	+13.6
F. May	27	52.4	161	84.2	96	63	56	53	206	206	205	932	+1.4
M. Canul	24	58.6	158	85.1	98	53	50	54	216	217	230	960	+2.3
G. Chan	28	45.9	146	81.2	95	46	43	46	190	183	181	944	+3.5
T. Ek	27	57.2	160	84.4	99	39	35	34	212	216	210	929	+0.4
J. Cene	25	48.1	151	81.6	95	52	46	51	188	206	199	967	+4.4
G. Ek	37	56.3	163	88.5	93	48	41	41	235	238	226	1,012	+15.1
G. Chi	24	57.8	154	81.5	101	51	46	49	234	219	212	987	+4.8
C. Mucul	25	53.9	149	83.3	98	59	60	54	263	254	237	1,188	+26.1
R. Dzib	40	62.2	165	86.5	98	53	48	47	256	230	232	990	+12.6
M. Dzib	19	64.0	167	88.0	98	65	55	52	256	263	259	1,048	+8.9
B. Tun	31	52.2	155	82.1	98	50	47	48	238	220	217	1,049	+15.8
S. Mex	22	59.5	162	86.8	96	52	50	51	220	205	216	911	−4.0
A. Castro	30	51.8	149	80.1	100	48	46	43	208	221	221	1,038	+13.9
F. Ceme	28	52.2	156	83.0	97	48	50	51	215	209	204	970	+5.8
I. Mex	28	52.2	158	86.0	94	55	53	54	215	211	212	978	+6.7
L. Mex	39	62.6	164	89.2	96	80	73	80	249	251	259	1,046	+18.5
J. Puc	22	54.0	150	81.2	100	60	58	54	228	219	231	1,061	+11.3
D. Dzib	31	51.8	153	84.3	95	47	47	45	217	224	226	1,044	+15.7
A. Dzib	27	54.9	156	82.9	99	55	50	47	243	228	224	1,045	+13.4
F. Tun	36	53.1	155	83.0	98	46	47	47	203	197	212	945	+6.6
Average	27	54.4	156	83.5	98	54	51	52	222	219	220	1,001	+8.4

* Average room temperature during experiments, 21.0°C.; average outdoor temperature, 20.4°C.; the mouth temperatures showed, if anything, somewhat subnormal values.

** M. Dzib also measured on a fourth and fifth day; pulse rate 50 and 54; O_2, 173 and 178 cc.; deviations from prediction, −12.5 and −10.3 per cent.

and stocky. Although it may be argued that Pirquet's standards may not be directly applicable to the Maya, it is evident that there is no obvious disproportion in the body configuration of these men and that, as a whole, they may be classed as well-nourished individuals, not under or over weight. It is particularly important to bear this fact in mind in our subsequent consideration of the metabolism measurements themselves.

The stem length as an index of normality of body form. According to Pirquet (4) the sitting height of an adult white person may be calculated by dividing the measured standing height in centimeters by 2 and adding 5 cm. Obviously with those individuals having a long stem length this standard correction of 5 cm. will be too small. Hence if the difference between the actually measured sitting height and half the standing height is calculated, one can get some hint as to the normality in the general configuration of the individual. For our group of 30 Maya this difference has been calculated and found to range from 2.6 cm. with T. Canul to 8.8 cm. with C. Mucul. The average difference is 5.6 cm. It is safe to assume, therefore, that the stem lengths of these Maya are not abnormally long and that their body configuration is not greatly different from the general configuration characteristic of a group of Whites.

Pulse rate. The average pulse rates, based upon the several observations on each of the three days of experimentation with each subject, are also given in table 1. The average rate of this group of 30 men was 54 beats per minute on the first day, 51 on the second, and 52 on the third. Judging from this evidence alone, one could assume that there was no great difficulty in establishing a normal state of mental and muscular repose with these subjects, even with the novelty of the experimental procedure. There are notable instances where the pulse rate is 45 or below. Thus G. Chan has an average rate for the three days of 45, T. Ek of 36, and G. Ek of 43. A. Castro has a rate on one day of 43 and D. Dzib on one day of 45. The most striking case is that of T. Ek, whose pulse rate on the three successive days was 39, 35, and 34 beats per minute, respectively, values measurably lower even than the low rates noted for this same man by both Williams and Shattuck. In the Williams and the Shattuck series, although there are no pulse rates below 40, there are a number between 40 and 45. It is an established fact, therefore, that these adult Maya not infrequently have a very low pulse rate. If the grand average is calculated of all the records of pulse rate noted with the Maya on this third expedition, these Indians may be considered to have a pulse rate of 52 beats per minute. This grand average represents a large number of counts on three days in the case of each man. The more limited data obtained in Williams' series show an average rate of 55 beats, and those observations reported by Shattuck an average of 56 beats. Of the 136 white men whose basal metabolism measurements have been listed by Harris and Benedict (5),

the pulse rate was recorded for 121 individuals and found to average 61 beats per minute. It would seem, therefore, that the Maya as a whole have a low pulse rate. It is regretted that studies of the heart rate with electrocardiograms could not have been made, particularly in the case of T. Ek. For normal Whites, without heart block, no records of such low pulse rates exist except in those instances noted by Miles (6) with men who had been living on a sub-maintenance diet. Pulse rates as low as 35 were frequently found during the period of undernutrition, and one man had a rate of somewhat under 30. This phase of Maya physiology is worthy of further study, but obviously such study can not be carried out to advantage in the field.

Oxygen consumption. The oxygen values reported in table 1 are averages of three well-agreeing periods on each of three consecutive days. With some of the subjects the average for the first day is higher than the averages for the second and the third days. This is not uniformly the case with all the subjects. In general the oxygen consumption averaged 222, 219, and 220 cc. on the first, second, and third days, respectively. There is no great difference in these averages, and one could not conclude from these results that there is a tendency for the metabolism to become lower in the successive measurements, as was thought possible by Shattuck and Benedict.

Heat production per square meter of body surface. The heat production has been calculated from the average oxygen consumption noted on the three days and, solely because of custom, has been expressed per square meter of body surface per 24 hours. In this calculation the Du Bois height-weight chart (7) has been employed for estimating the body surface, on the assumption that this chart applies with a sufficient degree of accuracy to the Maya type of configuration. The average heat production of the 30 Maya is 1001 calories per square meter of body surface. The values for the different subjects are strikingly uniform. It is doubtful whether greater uniformity would be found with any group of 30 individuals, whether students, men off the street, or men from any group of workers. Thus, the minimum heat production is 911 calories with S. Mex, and the maximum is 1188 calories with C. Mucul. If C. Mucul is omitted, the maximum is 1069 calories.

Deviation of measured from predicted metabolism. The total heat production of these 30 men has been compared with the Harris-Benedict predictions (5) for white men of the same age, weight, and height, and the percentage deviations of the actually measured metabolism from the predicted metabolism are recorded in the last column of table 1. The majority of the percentages are plus. There are only four minus values and, of these, two are about −4.0 per cent and the other two are less than −1 per cent. Of those subjects having a higher metabolism than the prediction, C.

Mucul shows the greatest deviation, +26.1 per cent, and eleven of the other 29 men give values of over +10 per cent. The average for all 30 men is +8.4 per cent. This particular group of Maya, therefore, had a metabolism definitely higher than the North American standards, even higher than that noted in the two earlier Yucatan studies, for Williams reported an average deviation of +5.2 per cent and Shattuck of +5.8 per cent. This distinctly high metabolism was found not on one day but on three consecutive days.

The heat production of the 30 Maya studied on this third expedition was likewise predicted from the Aub and Du Bois standards (8), although the calculations have not been incorportated in table 1. The average percentage deviation of the actually measured metabolism of this group of Maya from the metabolism as predicted by the Aub and Du Bois standards is +5.1 per cent, as compared with the +8.4 per cent when the prediction is made by the Harris-Benedict standards. In both cases the metabolism is definitely plus.

Our subject M. Dzib had been in the United States for 2½ years just previous to these metabolism tests. F. Echemaria, during the time of these observations, was fed at the mess of the Carnegie Institution of Washington and hence was not partaking of native food. These two men show essentially the lowest metabolism in the entire series, but we would not definitely ascribe their low metabolism to either of the above-mentioned facts. This information is recorded, however, as of possible later use in any subsequent studies of the effects of climate and food.

In this third expedition it was possible to study 12 of the same men measured by Shattuck. Seven of these men had likewise served as subjects for Williams. The percentage deviations (from the Harris-Benedict standards) found with these men by the three different investigators are recorded in table 2. The average deviation for the 12 men was found by us to be +7.2 per cent and by Shattuck to be +3.3 per cent. For the 7 men measured in all three expeditions, the deviations averaged +5.7 per cent in the Williams series, +1.5 per cent in the Shattuck series, and +8.1 per cent in our series. In comparing the metabolism as measured by us with that measured by either Shattuck or Williams, one should bear in mind that in our series the averages are in every case the result of three days' observations comprising nine periods in all, whereas in both of the earlier series the values represent frequently only one day or two days and oftentimes only two periods on any given day. We are convinced that some of the birth dates furnished to Shattuck were erroneous and that a number of his subjects were older than recorded in the Shattuck series. This applies likewise to a certain extent to the Williams series. The metabolism predicted by the Harris-Benedict standard for a man of 21 years, for example, will be higher than that predicted for the same man with

an age of 25 years. If his measured metabolism is above the predicted metabolism for the age 21 years, it will therefore be even higher above the prediction for 25 years. This probably explains in part the differences in the average percentage deviations noted for the same subjects in the three expeditions. When it is considered that these three series of measurements were made at intervals of from one to three years, it is surprising that the agreement on the whole is so uniform.

TABLE 2

Comparison of metabolism data secured on the same male Maya by three different investigators

SUBJECT	DEVIATION OF MEASURED FROM PREDICTED (HARRIS-BENEDICT) METABOLISM		
	Steggerda	Shattuck	Williams**
	per cent	*per cent*	*per cent*
E. May*	−0.2	−2.2	±0.0
T. Canul	+7.5	+7.5	
E. Puc	+9.7	+1.7	+6.1
S. Ceme	+12.1	+11.5	+3.3
J. Ceme	+8.7	+6.4	
D. Balam*	+13.6	+11.8	
J. Cene	+4.4	+6.6	
G. Chi	+4.8	+1.1	+11.5
C. Mucul	+26.1	+8.7	+13.7
G. Chan	+3.5	−8.5	+5.1
T. Ek	+0.4	−1.8	−0.1
S. Mex	−4.0	−3.4	
Average	+7.2	+3.3	
Average for same 7 men studied by Williams	+8.1	+1.5	+5.7

* Erroneously recorded as U. May and D. Balm in the report by Shattuck and Benedict (2).

** In the report by Williams his subjects were not indicated by names but by Roman numerals. Those subjects listed in this column, in the order as listed, were assigned the following numbers in his paper: XXI, XXVIII, XXXVI, XXIII, XXIX, XX, and XXXIX, respectively.

General considerations. The general experience of the Nutrition Laboratory has been that with one and the same individual, when a relatively low pulse rate prevails, the metabolism is, in general, somewhat lower than when a relatively high pulse rate prevails. But this thesis deals only with the individual and not with the group. Hence from this standpoint alone no correlation between the average pulse rate of the group of Maya as a whole and their average metabolism is to be expected. Examination of the data for any one subject in our series shows that the

pulse rates for the most part are fairly uniform on the three different days. When material differences occur, there is occasionally a lower metabolism simultaneously with a lower pulse rate, but for the most part there is no correlation between these functions. Unless the heart rate may be taken as a direct measure of blood flow, obviously no correlation can be expected. It is conceivable, however, that with the Maya, as distinguished from most of the Whites, the volume output of the heart may vary greatly under different conditions. But it is clear that the heart rate and the total metabolism are not correlated in the individuals in this group. Indeed, of the 11 subjects having unusually low pulse rates, averaging 49 beats per minute or below, not one has a metabolism below the Harris-Benedict standard. All but one are above the prediction standard by 3.5 per cent or more, and five of them are more than 10 per cent above.

Had this high metabolism been noted in the case of a few Maya only, one might consider that it was but a chance observation. We believe that it has been established beyond question, however, that male Maya have a pronouncedly high metabolism and, in most instances, a low pulse rate. The reason for this high metabolism is difficult to understand. It has challenged attention from the time of Williams' first report, and the fact that Williams' observations were repeated by two later expeditions in the attempt to confirm or refute his measurements demonstrates that this finding (confirmed by all three expeditions) has real importance in the general study of racial metabolism. Dietary studies, particularly a study of the total nitrogen output per day, should be made with this most interesting people. A cursory survey of the food habits of the Maya leads to the belief that these men were on a distinctly protein-poor, non-stimulating diet. The factor of a protein-rich diet, therefore, and furthermore such factors as the bustle and drive typical of an active metropolitan center and extreme athletic prowess, which are known to stimulate metabolism, did not obtain under the conditions noted in Yucatan.

These Maya were living in a sub-tropical climate, under semi-primitive conditions. Climatological records for the years 1928 to 1931 show that the *minimum* temperatures in Chichen Itzá, when the experiments with the Maya during the second and third expeditions were made, were on the average as follows: January, 14.5°; February, 14.8°; March, 16.3°; and April, 18.2°C. In not a few instances during the third series of observations, temperatures at 6 a.m. of 8° to 10°C. were actually noted. Based upon experience in Western Cuba and his endocrinological studies, our colleague, Dr. Oscar Riddle[2] of the Department of Genetics of the Carnegie Institution of Washington, has emphasized the possibility that we have to

[2] We are very grateful to Doctor Riddle for his repeated, painstaking reviewing of this manuscript and for his keen, helpful criticisms.

deal here with a temporary adjustment of the thyroid to offset the effects of extremely cool, if not cold nights. The Maya sleeps inadequately protected by his meager clothing and his hammock against these cold nights. The majority of Williams' observations, however, were made in July, when the average *minimum* temperature is more nearly 20°C., and yet his July measurements show a definitely high metabolism. We are therefore convinced that in this high metabolism we are dealing primarily with a racial characteristic, although we have not as yet completely disposed of the possibility of a climatological factor.

The suggestion made in Williams' report that the metabolism is higher with the Indians having the purer Maya blood was challenged in the Shattuck paper. Since these two articles have appeared, a monograph has been published by Williams (9) in which his original assertion is still maintained. Our anthropometric studies convince us that Williams' thesis is definitely strengthened by our observations. We believe that our subjects were of an even purer Maya strain than his, which may in part, at least, account for the fact that the average percentage deviation in metabolism of our subjects is higher by 3 than Williams' average percentage deviation.

Just prior to the preparation of this present report there appeared another paper published in connection with the comprehensive program of the Nutrition Laboratory in the study of racial metabolism, namely, the account of the observations made by Prof. Eleanor D. Mason of the Women's Christian College at Madras, India, on the basal metabolism of native South Indian women (10). Fifty-four young women students (chiefly Tamils, Malayalis, and Telugus) served as subjects. The metabolism was observed in all but one instance on at least two days, comprising from 4 to 6 periods, and frequently many more. The pelidisi of these young women did not indicate an abnormal condition or state of undernutrition, and yet their metabolism on the average was 17 per cent below the Harris-Benedict predictions. It is of interest to compare this group of South Indian women with the group of male Maya, since they have such distinctly different metabolic levels. The group of 30 male Maya were found to have an average heat production of 1001 calories per square meter of body surface per 24 hours. Among the South Indian women a group of 27 Tamils had an average surface area, by the height-weight formula (7), of 1.39 sq. m. and an average 24-hour heat production of 753 calories per square meter of body surface. On this basis the metabolism of the male Maya is 33 per cent above that of the female Tamils.

At the time of the formulation of the surface-area law, no consideration was given to differences in sex and age, and certainly none to differences in race. Among these Maya and Tamils there is a slight difference in age, since the Maya average 27 years and the Tamils 21 years. But the most

important factors are sex and race. According to the Aub and Du Bois prediction standards, women are considered to have a metabolism 7 per cent lower than that of men. The experience of the Nutrition Laboratory leads us to believe that the difference in the heat production of men and women is somewhat greater than this (11). By no process of reasoning, however, can this difference of 33 per cent between the metabolism of the Tamils as a group and that of the Maya as a group be ascribed wholly to the influence of sex. In the earlier pronouncement of the surface-area law (12), values were given for the heat production of various animals per square meter of body surface, ranging from 1188 calories with the mouse to 917 calories with the rabbit. The metabolism of the mouse was 30 per cent higher than that of the rabbit. Thus, as is seen from the comparison of the metabolism of the Maya and the Tamils, the variability in the metabolism of humans is somewhat greater than it is within the group of various animals whose metabolism data were used for formulating the surface-area law. Furthermore, the animals were studied under conditions where thermic neutrality did not exist. With humans a condition of thermic neutrality does obtain and the basal metabolism is therefore, strictly speaking, more nearly the true basal. The fact should therefore not be lost sight of, particularly in the use of basal metabolism predictions and in basal metabolism studies, that of two groups of humans in thermic neutrality, one group may have a heat production per square meter of surface area 33 per cent above that of the other group. This difference can be attributed only in small part to differences in age and sex; it may be due partly to environment, but there is a good deal of evidence to indicate a racial effect.

SUMMARY

A third expedition to Yucatan confirms the findings of two earlier expeditions, that the male Maya have a high basal metabolism and a low pulse rate. A group of 30 male Maya had a heat production on the average 8 per cent above the prediction standards for Whites. Anthropometric measurements indicated that these Maya were well nourished and had a configuration not greatly different from that characteristic of Whites. The average pulse rate was 52 beats per minute. With one subject rates as low as 34 to 39 were noted, and with four others rates of about 45. The possibility of great intra-specific differences in metabolism is shown by the fact that the heat production of this Maya group was 33 per cent above the measured metabolism of a group of 27 South Indian women. This difference in metabolism can be attributed only in small part to the influence of age and sex and in somewhat larger part to the influence of environment, but the major part of the difference must be considered indicative of a real racial effect.

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INSULIN IN TISSUES OTHER THAN THE PANCREAS

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A review of the literature, in which the distribution of insulin or insulin-like substances has been discussed, reveals the fact that numerous investigators have reported the presence of these active materials in a great variety of animal and vegetable tissues. With the procedure originally used to obtain insulin from the pancreas, insulin was not obtained from other tissues (Banting and Best, 1922). It was perhaps not surprising that the improved methods of extracting pancreatic insulin when applied to other animal tissues should apparently yield definite amounts of active material (Collip, 1923; Best, Scott and Banting, 1923; Ashby, 1923; Baker, Dickens and Dodds, 1924; Best, Smith and Scott, 1924; Ivy and Fisher, 1924; Lundberg, 1924; Brugsch and Horsters, 1924 and 1930; Nothmann, 1925; Cori, 1925; Vincent, Dodds and Dickens, 1925; Penau and Simonnet, 1925; Hoshi, 1926; Cramer, Dickens and Dodds, 1926; Redenbaugh, Ivy and Koppanyi, 1926; Shikinami, 1928). Certain points in these reports on the distribution of insulin stand out very prominently. It is surprising that insulin should be reported to be present in diabetic tissues, (Best, Smith and Scott, 1924; Baker, Dickens and Dodds, 1924; Nothmann, 1925; Pollak, 1926) and in relatively large amounts in the zymogenous tissue of teleosteal fishes (Vincent, Dodds and Dickens, 1925) and in spleens of normal rats (Cramer, Dickens and Dodds, 1926). Furthermore, the urinary secretion of very large amounts of active material after the intravenous or oral administration of insulin, or after its introduction through an intestinal fistula (Fisher and Noble, 1923) and the peculiar behaviour of "endogenous" and "exogenous" insulin (Brugsch and Horsters, 1924, 1930), are very odd phenomena.

The apparently wide distribution of insulin-like material in the animal kingdom led Collip to suggest that this material might be present wherever glycogen was formed. The active material was, however, later thought to be present in extracts of plants, in the tissues in which no glycogen can be detected (Collip, 1923). Collip also encountered a strange phenomenon of "animal passage" of the hypoglycemia-producing principle. Later he suggested (Collip, 1927) that the passage phenomenon was probably attributable to a "living agent." It is obviously impossible to decide from

the evidence at present available how much of the delayed hypoglycemia observed after the injection of extracts from vegetable sources was due to a chemical substance, and how much to the effects of the "living agent" which might have been present in the extracts. The evidence suggests that most of the changes observed by Collip were due to a contaminating "living agent." It must be borne in mind, however, that large doses of pancreatic insulin may produce a prolonged hypoglycemia after a preliminary period in which the blood sugar is not lowered. It is perhaps significant that some of the workers with plant extracts secured prompt hypoglycemia (Dubin and Corbitt, 1923; Best and Scott, 1923; Fisher and McKinley, 1924; and others). Collip (1923) secured only delayed effects from yeast extracts, while Hutchinson, Smith and Winter (1923) secured more rapid effects comparable to those produced by pancreatic insulin. In Collip's work on extract from clams, hypoglycemic convulsions were observed in rabbits within six hours. The possible rôle of pancreatic insulin in the production of some of these results will be clearer when the results we have recently obtained have been discussed.

There are a number of reasons why the results of the early work on the distribution of insulin and insulin-like substances are of no significance from the quantitative point of view. It was not then fully appreciated that some rabbits may show a definite fall in blood sugar over the experimental period, and for this reason the results based on one animal are not satisfactory. A standard of comparison was not used in the assaying of insulin in most of the early work. In a number of the papers which have appeared on the subject of the distribution of insulin, no convincing evidence is to be found that any hypoglycemia-producing substance was present in the extracts which were reported to yield positive results. When we add to these factors the possibility of the presence of a living agent which may cause hypoglycemia, the possibility that non-specific toxic materials, which may produce liver damage and delayed hypoglycemia, may have been present, and the possibility of contamination of solutions by pancreatic insulin, it becomes apparent that the whole question of the distribution of insulin and insulin-like substances must be reopened, and that many, if not all, of the results obtained up to the present time be discarded.

In the previous work several investigators have stated that they were able quantitatively to recover insulin added to pancreas or other tissues. Few, if any, of these results will, in the light of the present knowledge, stand critical analysis. In most cases one of the well known methods for making pancreatic insulin was used. In work of this kind it would be preferable to have available a method that would yield optimal results when applied to pancreatic tissue. With this point in mind, one of us (Jephcott, 1931) has thoroughly explored the conditions under which maximal yields of

insulin in a form in which it can be accurately assayed are obtainable from pancreatic tissue. The details of the procedure finally adopted are as follows: perfectly fresh glands are finely minced, and after thorough stirring 25 gram samples are quickly weighed and transferred to a flask containing 75 cc. of 95 per cent ethyl alcohol, 25 cc. of water, and 1.5 cc. of concentrated hydrochloric acid. Extraction is carried out in a mechanical shaker immersed in a water bath which is kept at a temperature of 36°C. After two hours the mixture is filtered through a double thickness of gauze and the residue is pressed as dry as possible. A second extraction, similar to the first, is then made. The two filtrates are combined, made just alkaline to litmus with ammonia, filtered through a Buchner funnel and re-acidified to pH2 with hydrochloric acid. The alcohol is distilled off under vacuum at about 35°C. The residue is transferred to a 250 cc. measuring flask and made up to volume with water. When desirable the active material may be salted out with ammonium sulphate or sodium chloride without appreciable loss. The assay on white mice and rabbits is conducted as soon as possible after the extract has been prepared. The assays are made by comparison of the effect of the unknown solution with that of standard insulin. After the potency of the solution is determined approximately, from thirty to sixty white mice and several rabbits are used for each test. When the procedure outlined above is used to extract insulin from beef pancreas, average yields of approximately 3000 units per kilogram may be consistently obtained. We believe that yields of 3500–4000 units per kilogram represent the maximal amounts of active material which can be secured by the methods available at present from beef pancreas collected in Toronto during the summer months. Higher yields reported by other workers are open to question since the methods of assay then used cannot now be considered adequate. The yields of insulin from the pancreas of various species have been determined by one of us (C. M. J.) and will shortly be reported.

It might be expected that no difficulty would be encountered in recovering added insulin with a method such as we have outlined. Such, however, is not the case. Purified insulin or the crystalline product cannot consistently be recovered quantitatively when the hydrochloric acid procedure outlined above is used. The results of numerous experiments demonstrate that an average recovery of more than 60 per cent of the added material is not usually secured. When sulphuric acid is used instead of hydrochloric the recovery is not significantly improved. Lower yields of insulin from pancreas are always obtained when sulphuric acid is used.

It can be shown, however, that crude insulin withstands this treatment much better than the purer material (Jephcott, 1931). One of the factors affecting the destruction of the purified insulin may be the length of time

it is exposed to the alcohol and acid. Even when the tissue is extracted rapidly, however, a great deal of the added material may be lost. Insulin prepared by different procedures varies considerably in its stability under these circumstances.

The situation is, therefore, that the method which gives the best yield of insulin from pancreas does not consistently permit more than a partial recovery of purified insulin added to pancreas or to other tissues. When the hydrochloric acid procedure is used to investigate the insulin content of tissues other than the pancreas, negative results might conceivably mean that small amounts of insulin were present but were destroyed by the treatment. If, however, the hydrochloric and sulphuric acid extractions and various methods of purification are carefully applied and negative results are consistently obtained, the suggestion that demonstrable amounts of insulin or insulin-like materials are not obtainable from other tissues would be supported.

The results of the application of these procedures to vegetable tissues,

TABLE 1

TISSUE	WEIGHT	METHOD	MOUSE TEST	RABBIT TEST
	grams			
Onions	1,100	Alcoholic HCl	Negative	Negative
Beet roots	1,400	Alcoholic HCl	Negative	Negative
Beet roots	1,100	Alcoholic H_2SO_4	Negative	Negative
Beet roots	1,800	Alcoholic HCl	Negative	Negative

normal beef and dog tissues, to urine and to diabetic dog tissues will be briefly reported and discussed.

Vegetable tissues. It is unnecessary to review in detail the numerous reports on the presence of insulin-like substances in plant tissues (Collip, 1923 a, b, c, d, e; Funk and Corbitt, 1923; Dubin and Corbitt, 1923; Winter and Smith, 1923 and 1925; Hutchinson, Smith and Winter, 1923; Best and Scott, 1923; Gottschalk, 1924; Eisler and Portheim, 1924; Glaser and Wittner, 1924; Fisher and McKinley, 1924; Simola, 1927; Shikinami, 1928; Kaufmann, 1928, etc.). The results of these studies may be divided into three groups; *a*, in which there is no convincing evidence of the presence of any active principle; *b* in which there is proof that a delayed hypoglycemia occurs, and *c*, in which the hypoglycemia appears more promptly. If we make the obvious inference from Collip's suggestion that a living agent may be responsible for the "animal passage" phenomenon, that it might also account for the delayed hypoglycemia observed after the subcutaneous injection of vegetable extracts, and admit the possibility that contamination of the plant extracts by pancreatic insulin may be responsible for the more rapid effects, there would be no evidence suggesting an insu-

TABLE 2

TISSUE	AMOUNT	METHOD OF EXTRACTION	METHOD OF ASSAY	RESULT*
Arterial blood (dog); no general anesthetic	160 cc. serum	Alcoholic H_2SO_4	Mouse and rabbit	Negative
	205 cc. cells	Alcoholic H_2SO_4	Mouse and rabbit	Negative
Arterial blood (dog); no general anesthetic	540 cc. defibrinated blood	Benzoic acid	Mouse and rabbit	Negative
Splenic vein blood (dog); amytal anesthesia	57 cc.	Benzoic acid	Mouse	Negative
Dog's liver	230 grams	Alcoholic HCl	Mouse	Negative (toxic effects)
Dog's liver	100 grams	Benzoic acid	Mouse and rabbit	Negative
Dog's liver	150 grams	Picric acid	Mouse and rabbit	Negative
Dog's liver	75 grams	Fisher's method	Mouse and rabbit	Negative
Sheep liver	60 grams	Alcoholic HCl	Mouse and rabbit	Negative (toxic effects)
Sheep liver	100 grams	Picric acid	Mouse and rabbit	Negative
Beef liver	10 grams	Alcoholic HCl	Mouse	Negative
Dog's muscle	100 grams	Benzoic acid	Mouse and rabbit	Negative
Beef salivary glands	100 grams	Alcoholic HCl	Mouse and rabbit	Negative
Beef salivary glands	500 grams	Picric acid	Mouse and rabbit	Negative
Dog's heart	50 grams	Picric acid	Mouse and rabbit	Negative
Dog's heart	38 grams	Fisher's method	Mouse	Negative
Dog's kidney	47 grams	Alcoholic HCl	Mouse	Negative
Dog's spleen	35 grams	Alcoholic HCl	Mouse	Negative
Beef thymus	800 grams	Benzoic acid	Mouse and rabbit	Negative

* The possibility that the very potent but labile form of insulin reported by Dingemanse (Arch. Exp. Path. Pharm: 128, 44, 1928) and more recently observed by Dirscherl (Hoppe-Seyler's Ztschr. f. Physiol. Chemie, 202, 116, 1931) may have been responsible for any of the positive results previously obtained can, in our opinion, be regarded as extremely faint.

lin-like substance (glucokinin) in vegetable tissues. Collip (1927) apparently does not consider that the initial hypoglycemia produced by the vegetable extract is attributable to the same factors which cause the "animal passage" hypoglycemia.

When vegetable tissues were extracted by the procedure most successfully used to obtain pancreatic insulin, negative results were secured.

The extracts made from beet roots produced very toxic effects in several of the rabbits.

While sufficient experiments have not been performed to justify the conclusion that an insulin-like substance (glucokinin) is not present in plant tissues, it may be stated that in light of the recent findings the previous reports cannot be accepted at their face value. (The substances which may be extracted from members of the myrtle family, and which are reported to produce an antidiabetic effect when given by mouth, are not considered in this discussion.)

Normal animal tissues. A great many experiments have been conducted in this part of our work. A limited number of typical results will be reported. In addition to the methods we have discussed above, the exact procedures advocated by Fisher and by Baker, Dickens and Dodds and by others have been used.

Diabetic tissues. The failure to demonstrate insulin in normal tissues other than the pancreas made it extremely unlikely that any antidiabetic principle would be found in diabetic organs. We have taken advantage of an opportunity to secure diabetic tissues, however, and these have been analyzed by two methods (sulphuric and hydrochloric acid extraction) in an attempt to secure insulin. Liver, skeletal muscle and heart muscle were removed from a completely depancreatized dog which had received no insulin for 5 days. The results of the assay of the extracts prepared were completely negative. It does not follow from these results that the pancreas of a patient dying in diabetic coma contains no insulin, but a reinvestigation of this subject is perhaps necessary.

Insulin in urine. A hypoglycemia-producing substance has been reported to be present in urine by several groups of workers (Best, Smith and Scott, 1924; Kozuka, 1927; Partos, 1929; Brugsch and Horsters, 1930). This aspect of the subject of the distribution of insulin has been particularly developed by Partos and by Brugsch and Horsters. Recently Laurence, Modders and Millar (1930) have reported that they are unable to confirm the results obtained by these investigators.

We have reinvestigated this field and have been unable to secure demonstrable amounts of insulin from normal dog or human urine.

Recovery of injected insulin. The urinary excretion of injected insulin (Fisher and Noble, 1923) needs further comment. These investigators reported that large amounts of insulin could be recovered from the urine

of normal dogs after the oral or intravenous administration of the active material. Soon after this work was published R. G. Smith in our laboratory attempted to repeat it. In his first experiments he was apparently successful but later in numerous experiments was unable to detect more than traces in the urine even after the oral or intravenous administration of enormous doses to dogs. So many possible variables were uncontrolled in these experiments that the results were not reported at the time. Quite recently we have repeated certain of these experiments with the following results. When an insulin solution is administered intravenously to a dog under amytal anesthesia at such a rate that 200 units are given in the first two hours it is found that less than 10 per cent of the injected insulin can be recovered from the urine voided during the four hour experiment. Saline was given during the last two hours of the experiment. The urine was acidified and immediately tested for insulin by injection directly into mice and rabbits. No insulin could be recovered by the benzoic acid method from liver, kidney, muscle or heart tissues which were removed at the end of the experiment. These results demonstrate that, although most of the injected insulin was lost, a small but definite amount can be recovered from the urine under the conditions of these experiments. In a few experiments in which large amounts of insulin were administered by mouth to normal dogs, none was recovered in the urine.

Discussion. It is necessary to define what is meant by negative result in the assay of solutions for their insulin content by the mouse and rabbit methods. As it is not advisable to administer more than 0.5 cc. of solution to a mouse, the volume of all extracts was kept as small as possible. From 0.25 to 0.50 cc. was injected. When no symptoms attributable to the presence of insulin are observed in any of the injected animals it is safe to conclude that less than 1/100 unit of insulin is present. As approximately 25 cc. of solution were obtained from 100 grams of tissue a negative result indicates that less than ½ unit was present in the extract from this amount of material. Mice may occasionally exhibit convulsions which resemble those produced by insulin when tissue extracts which do not contain demonstrable amounts of this active material are injected. It is sometimes difficult to decide whether or not the administration of dextrose solution improves their condition. Repetition of tests, the results of which are difficult to interpret, and comparison with the results of the rabbit assay, however, always enable an investigator to make a correct interpretation of these doubtful results. Rabbits are injected subcutaneously with from 10 to 15 cc. of solution. A blood sugar lowering of from 30 to 40 mgm. per cent when the normal value is high (0.13 to 0.15 per cent) is not proof of the presence of insulin. It is inadvisable to conclude that insulin is present if very large doses of the extract under test do not

lower the blood sugar of some of the test rabbits fairly rapidly to the convulsive level.

One investigator (F. A. Calderone, who was working under the direction of Prof. G. B. Wallace) has informed us that he has been unable to confirm the results of the workers who reported the presence of insulin in blood (Best, Scott and Banting; Nothmann; Hoshi) but as far as we know there have been no published reports to this effect. Until we had secured the negative results reported above, we were inclined to attribute these apparent difficulties to the same factors which, in the early stages of the work on insulin, made it impossible consistently to obtain active material from pancreatic tissue. The demonstration of insulin in blood by physiological methods (Zunz and La Barre, 1927–29) seems to be convincing. The real situation very likely is that suggested by the observations of Kepinow and Petit-Dutaillis (1927) and of Heymans (1927). Insulin added to blood kept at body temperature was found to disappear very rapidly. In the light of our recent observations we believe that there is no evidence that any tissue other than pancreatic either produces or stores insulin. It is, of course, possible that methods will be developed which will permit the recognition of the minute amounts of insulin that one would expect to be present. Although we have not attempted to repeat in detail the extensive work of Brugsch and Horsters, from the results of which they conclude that injected insulin liberates endogenous insulin, etc., etc., we suggest that their results may be different if the experiments are repeated, using the precautions suggested by our experimental results.

A great deal of space might be utilized in the further discussion of the results which we have been unable to confirm. Many of these considered to be positive are obviously attributable to what we now know to be faulty interpretations of the results of the assays. The possibility exists that a contaminating living agent may have been responsible for the positive results in some cases. There is no doubt, however, that the active material found to be present in extracts of these various tissues by several groups of workers was insulin. It is perhaps significant that in Toronto, and in Collip's laboratory, large amounts of insulin were being prepared from pancreas. In Dodds' and Dickens' laboratory, work on the preparation of insulin was also in progress. In the laboratories where Fisher and Ashby, and Lundberg were studying, interest in the preparation and purification of insulin was being taken at the time the work referred to above was done. Although insulin clings tenaciously to laboratory glassware, etc., the usual cleaning procedures carefully applied are sufficient to remove the last traces. If contamination of solutions did occur, it is obviously impossible to make any rational suggestion concerning the mechanism.

It is regrettable that a great deal of what appears to be useless work has been done in this field. The results reported in this paper, in our opinion,

serve to remove from serious consideration many reports which made it difficult to accept the pancreas as the only source and the only important storehouse of insulin in the animal body.

SUMMARY

Using a method of extraction which yields maximal amounts of insulin from pancreas, we have been unable to demonstrate the presence of an antidiabetic substance in other normal tissues from the dog or ox or in the tissues of diabetic dogs. Positive results previously reported we believe can be attributed to one or more of several factors, each of which is discussed in this communication. Certain experimental results which have indicated a wide distribution of an insulin-like substance in the plant kingdom have not been confirmed.

We have had the opportunity of discussing these results with Prof. E. C. Dodds and Prof. A. C. Ivy. These investigators have repeated certain of the experiments performed in their laboratories, the results of which suggested that insulin or an insulin-like substance could be obtained from mammalian tissues other than the pancreas. The results of these recent experiments confirm the negative findings reported in this paper.

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THE EFFECTS OF SYMPATHETIC DENERVATION OF MAMMALIAN TISSUES DURING THE PERIOD OF POST-NATAL GROWTH

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A series of studies, to which the author has contributed (1931), has sought in vain, in the adult of mammalian species, for a trophic influence exerted by the sympathetic nervous system over non-nervous tissues in general. In particular, skeletal muscle shows no structural alteration following sympathetic denervation. However, the life which an adult animal leads in a laboratory—confined in a cage, fed and protected,—provides perhaps, an inadequate test for a tenuous factor in trophic control such as the sympathetic nervous system has been thought to supply. Cannon (1930) and his students found, indeed, that their completely de-sympathectomized animals could survive only under such carefully regulated conditions. However, it is common experience that the same life in a laboratory presents grave difficulties to the young of a species. Therefore, development of young animals under these conditions seemed to offer a most stringent trial of the importance of the sympathetic nervous system in nutritional or metabolic regulation of non-nervous tissues. With this in mind, the present study was undertaken.

Material: methods. A series of kittens was operated on between the seventh and tenth days of life. Two operations were performed. In one group of animals, the right stellate ganglion and thoracic sympathetic trunk were removed down to the 4th thoracic interspace. Of these animals, six survived. In a second group, in addition to the operation just described, the entire right cervical sympathetic chain was also resected, ganglia, trunk and ansa subclavia. Of these, four survived.

The members of each group were killed at the expiration of allotted times; six weeks, three months, six months and a year. The two extra animals of the first group were kept for the longer periods,—six and twelve months. The animals were killed with ether, the blood vessels washed out with physiological salt solution, portions of muscle removed to assorted fixatives, and the entire animal injected with 80 per cent alcohol.

At autopsy the size and development of corresponding structures on the two sides of the body were compared. Specimens were removed and weighed. Finally, histological preparations were made, utilizing the

Bielschowsky silver technique for muscle, Einarson's (1932) new gallocyanin technique for sympathetic ganglia, the Ranson-Cajal technique for sympathetic nerve trunks, and iron hematoxylin for all other tissues. Ranvier's gold chloride technique was also applied to muscle, for the study of the perivascular nerve plexuses.

Observations. The search for a so-called trophic influence exercised by the sympathetic nervous system on non-nervous tissues during the crucial period of growth and maturing of the animal, proved entirely fruitless. In the living animal, the development of the fore limbs, and indeed, of the entire fore half of the body, was quite symmetrical. The muscles, in bulk and strength; the bones, in size and hardness; the skin, hair,—all showed no effect of the prolonged sympathetic denervation. Only the complete and permanent loss of sudomotor and vasomotor function on the side of operation, and the Horner's syndrome, gave evidence of the lesion, and index to the state of repair.

The weights of specimens taken at autopsy from the four animals with complete thoraco-cervical sympathectomy, also showed negligible differences between the operated and normal sides. These specimens comprised the halves of the brain, the palmaris longus and extensor digitorum lateralis muscles, and the lateral lobes of the thyroid gland. One defect only was noted at autopsy, other than the absence of the sympathetic tissue removed at operation. The right superior cervical ganglion was consistently smaller than the left in the six cats in which it remained. (A series of six normal cats established the practical equality in size of these two ganglia, ordinarily.)

Examination of the microscopic preparations confirmed the gross observations. The Vth interosseous muscles were very carefully compared. But neither size of the muscle fibers, nor any characteristic such as striation, nucleation, staining reaction or granulation, betrayed the presence in the right muscles of a lesion of any sort. The smaller intra-muscular arteries and arterioles, which are supposed to be particularly under the influence of sympathetic innervation, were thoroughly inspected. Yet even they showed no effect of presumably complete denervation, other than the absence of perivascular non-medullated nerve fibers. The thyroid glands, finally, also gave no indication, by size of follicle, character of epithelium, quantity or staining quality of colloid, of a different functional state on the side of lesion and on the normal side.

Uniformly then, the non-nervous tissues examined, failed to react to, or compensated entirely for sympathetic denervation. Both grossly and microscopically, they had attained normal size and form quite without the influence of sympathetic innervation. That the tissues examined were indeed sympathetically denervated, that there had been no regeneration of preganglionic fibers to take over the work of the completely excised

postganglion neurone, nor diversion of postganglionic neurones from adjacent regions to supply the denervated area, there is abundant evidence. For the fore limb, first, careful dissection at autopsy disclosed no gross structures open to interpretation as sympathetic fibers passing to the brachial plexus. In agreement with the autopsy findings, sudomotor and vasomotor functions never returned to the paw-pad. Moreover, when the methods applied by Tower and Richter (1931, 32) to the study of sympathetic regeneration were used on the group of animals surviving operation a year, the skin-resistance of the right fore paw-pad was found to be in excess of the normal maximum for a cat, and the skin-action-currents (galvanic skin response), which are the most delicate indicator of sudomotor innervation, were totally wanting. Finally, as previously recorded, the blood vessels, whenever examined, were found to be depleted of non-myelinated nerve fibers on the side of lesion. On the other hand, the gross form of the ansa subclavia was more or less restored in all ten animals, by strands passing from the first two thoracic nerves. In the animals with inferior and superior cervical ganglia intact, these strands contained a great many nerve fibers. Especially was this the case six months or a year after operation, when functional recovery in the eye was far advanced. In the four animals with complete cervical sympathectomy, however, sections of these strands just central to their point of junction with the vagus, showed the structure to be largely connective tissue, containing only a very few tortuous nerve fibers. And in these animals, after a certain recovery of tone during the first month, Horner's syndrome was static. Here, most clearly, there was no functional regeneration or preganglionic fibers down the postganglionic path, nor crossing over of fibers from the normal side to supply the denervated region.

Although the non-nervous tissues affected by the lesion showed no effect of prolonged sympathetic denervation, the cervical sympathetic ganglia, superior and inferior, cut off from the central nervous system for a variable time by resection of the stellate ganglion, showed most marked reactions. In the inferior cervical ganglion the reaction took the form of an apparent slight hypertrophy, both gross and microscopic. There was an increase in the number of cells of the types normal to the ganglion. However, this ganglion varies exceedingly in size, being in some animals, microscopic, in others grossly well developed. Hence, little importance can be attached to observations in so small a series as six cats. In the superior cervical ganglion a consistent small gross size of the right ganglion was reflected microscopically in a greatly diminished number of nerve cells. The cells also averaged somewhat smaller and the connective tissue elements showed an increase, perhaps only relative. The reduction in number and size of the cells seemed to affect all the range of sizes and types in proportion to their normal representation. For example, the very large cells

(40 $\mu \times 70\ \mu$), which appear only after the first half-year of life, were present on the two sides in proportion, but ran a trifle larger on the normal side. A more conspicuous difference between the cells of normal and decentralized ganglia than was afforded by size or form, was the noticeably larger size of the nuclei and their predominantly eccentric or polar position, on the size of lesion. Furthermore, the cytoplasm of these cells appeared to shrink in fixation excessively, a characteristic reported by Sternschein (1922). However, the chromidial substance of nucleus and cytoplasm showed no disturbance on the side of lesion. Nor were pigment granules, a normal cytoplasmic constituent of many sympathetic cells, increased in amount as Graupner has described (1898) for conditions of atrophy of sympathetic ganglia.

Conclusive evidence to indicate the cause of the diminished cell count on the side of operation was wanting. Of cellular degeneration there was no sign, even in the specimen taken six weeks after operation. Since the operation decentralizing the superior cervical ganglion was performed before the cervical sympathetic had assumed function (Horner's syndrome develops between the second and third weeks of life) and probably before the complement of cells has been fully made up, and since, moreover, the differences between the right and left ganglia were the greater, the longer the time elapsing after operation, it seems likely that the deficiency in the decentralized ganglia was a matter rather of incomplete development than of degeneration. On the other hand, resumption of function by the superior cervical ganglion did not correct the defect. Recovery from pupillary paralysis began in the sixth to eighth week, reached the stage of pupillary equality generally by the sixth to eighth month, after which a condition of over-dilatation supervened. Yet, the greatest differences between right and left ganglia were found in the animals sacrificed after a year, at which time the eye on the side of operation had been in a state of sympathetic over-action for months.

Conclusions. From the evidence presented, certain conclusions may be drawn as to the activities of the sympathetic nervous system. First, during the taxing period of development of the new-born into the adult, the influence of the sympathetic nervous system is not necessary for non-nervous tissues, in general, to attain normal size and form. At any time during the first year of life many such tissues, sympathetically denervated, are quite indistinguishable from the normal. This conforms with histological evidence the observations of gross form and size, reported by Cannon (1930) for kittens, and by McCullogh, McFadden and Milroy (1930) for puppies. Second, the evidence shows that even in the young animal, when presumably the impediment offered in distance and density of structure is minimum, preganglionic neurones do not replace the post-

ganglionic, although they are themselves growing rapidly and re-establishing their own connections across considerable gaps. Moreover, the neurones of the opposite intact side, or of levels below the lesion, are not mobilized to mend a sympathetic defect. Finally the evidence indicates that the influence of the sympathetic outflow from the spinal cord is essential to the proper development of the peripheral sympathetic ganglia. Contrary to the report of Anderson (1902), decentralized sympathetic ganglia fail to attain the gross size of normal ganglia, and their cell complement is less. The reduction in numbers of cells, and in their size, affects all elements of the cell population equally. No two groups of cells such as Dogiel (1896) has described, can be discriminated,—the one perhaps sensory and stimulated from the periphery, or, more likely, responding to intraganglionic stimuli, humoral or reflex, and preserved following decentralization; the other, dependent both for function and for existence entirely on the central nervous system. That a sympathetic ganglion and its connections exercise some variety of control over peripheral structures, in particular over smooth muscle, there is ample proof (Tower and Richter, 1932). But of a special type of cell serving such functions, the present study affords no indication.

SUMMARY

A series of kittens was operated on, between the seventh and tenth days of life, for removal of a greater or lesser portion of the right cervicothoracic sympathetic chain. The animals were allowed to survive a fixed period up to one year. During this time growth and development were carefully studied. At autopsy, corresponding normal and sympathetically denervated organs were compared and weighed. Finally, histological preparations were made and studied.

In the non-nervous tissues examined, sympathetic denervation was without appreciable sequel. Gross specimens of bone and muscle, of brain and thyroid gland from the side of operation and the normal, were quite alike in size, weight and form. Microscopically also, the specimens from the two sides were indistinguishable. The sympathetic ganglia cut off from the central nervous system showed, on the other hand, marked deficiency in gross size and microscopically, in cell content. The cells likewise showed certain morphological peculiarities.

The conclusions drawn were: 1, that sympathetic innervation is not essential to the growth or maintenance of non-nervous tissue in general; 2, that preganglionic fibers do not replace postganglionic, even in the very young animal, nor do sympathetic neurones deviate from their proper course to repair a defect; 3, that decentralization of sympathetic ganglia hinders the development of all postganglionic neurones so affected.

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THE EXCRETION OF URINE IN THE DOG

III. The Use of Non-metabolized Sugars in the Measurement of the Glomerular Filtrate

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No substance is known to be present normally in the plasma and urine of vertebrates which can be safely used to evaluate the quantity of glomerular filtrate under physiological conditions. Creatinine of exogenous origin has been recommended for this purpose by Rehberg, (1926) but when it is recognized that this substance is secreted by the renal tubules of the lower vertebrates (Marshall and Grafflin, 1932; Clarke and Smith, 1932) and that it is not present normally in significant quantities in the blood of mammals (Behre and Benedict, 1922; Gaebler and Keltch, 1928; Gaebler, 1930) its use is open to question.

We have sought for a foreign substance, practically and theoretically suitable for this purpose, among several groups of organic and inorganic compounds and have concluded that the non-metabolized sugars are satisfactory.

The properties which we believe such a substance should possess are as follows:

1. It must be determinable in plasma and urine with quantitative accuracy.
2. It must be non-toxic and it must exert no local stimulating or depressing action upon the kidney.
3. It must be completely filtrable from plasma.
4. It must not be secreted by the renal tubules.
5. It must not be reabsorbed by the renal tubules.

The choice of sugars for this purpose was suggested by the recent observations of Marshall (1930) that glucose is excreted by glomerular, but not by aglomerular fishes. On broad principles we relate this circumstance to the fact that, being a food and not a waste product, glucose has been invariably conserved by the vertebrates throughout their evolution and at no time continuously rejected from the body. The renal tubules have never been called upon, therefore, to secrete this substance, and are incapable of doing so. There is good evidence that this is equally true of

the mammals. The appearance of glucose in the urine of glomerular animals during hyperglycemia or after phlorizin is in this view an incidental phenomenon referable to incomplete tubular reabsorption of glucose from the glomerular filtrate. But because it is normally reabsorbed by the tubules, glucose itself is not suitable for the evaluation of the glomerular filtrate. Although this reabsorption can be paralyzed by phlorizin there is reason to believe that other physiological activities of the kidney are also influenced by this drug, so that its use is of dubious physiological value.

It seemed that if glucose is not secreted by the tubules, other sugars would not be secreted by them, and that among the metabolically inert sugars one or more might be found which was not reabsorbed. Our attention has been directed toward three which appear to be most suitable: the pentose, xylose; the disaccharide, sucrose; and the trisaccharide, raffinose.

1. *Quantitative determination.* The introduction of the rapid absorption of glucose by washed yeast cells as a method of determining the true glucose content of plasma and urine has made it possible to distinguish this sugar from other reducing sugars, such as xylose. When the concentration of xylose is maintained at a level of 70 mgm. per cent or higher in the plasma, the non-glucose reducing substances of dog plasma and urine can be neglected. (These average about 5 and 150 mgm. per cent, respectively, when using Somogyi's copper precipitation method.) Methods have been devised for the determination of either sucrose or raffinose in the presence of glucose by means of sucrase, for a full description of which the reader is referred to the section of this paper dealing with methods.

2. *Toxicity.* Xylose, sucrose and raffinose can be administered in large amounts without evidence of toxic action. The possibility of a local action upon the kidney will be referred to subsequently in the paper.

3. *The filterable character of the sugars.* There is no evidence that a combination exists in the plasma between glucose and protein, and although it is frequently suggested that the glucose in normal blood exists in some complex condition, there is ample reason to believe that in the phlorizinized animal the glucose is entirely filterable at the glomerulus—a conclusion which is supported by the experiments reported in this paper.

There is no reason to suppose that a physiologically inert sugar such as xylose can combine with protein or otherwise form non-filterable complexes, but to test this point we have filtered dog plasma containing 150 mgm. per cent of xylose through collodion membranes under a pressure of 100 to 150 cm. of Hg; the filtrate contained no protein, as shown by heating and the addition of trichloracetic acid, but the residue and the filtrate contained xylose and glucose in the same concentrations, within the limits of experimental error in the sugar analysis ($\pm$ 2 per cent).

4. *Xylose and sucrose are not secreted by the renal tubules.* We have already referred to Marshall's (1930) observation that the aglomerular

fish kidney is incapable of excreting glucose, even after large doses of phlorizin, indicating that glucose is excreted only by glomerular filtration. Jolliffe (1930) and Clarke and Smith (1932) have shown that xylose and sucrose, although freely excreted by glomerular fish, are not excreted by aglomerular fish from plasma levels of 300 to 400 mgm. per cent.

Though evidence derived from lower vertebrates should not be transferred directly to the higher animals, our rapidly growing knowledge of the comparative physiology of the kidney leads us to believe that sugars are never secreted by the renal tubules. Direct evidence, however, against the secretion of sugars by the renal tubules in the dog is now available in our observations, reported in this paper, that xylose and sucrose, and in the phlorizinized dog xylose and glucose, or raffinose and glucose, are excreted at the same rate relative to the plasma concentration; i.e., the respective *simultaneous glomerular clearances* for these substances are identical. This fact is consonant with filtration, but irreconcilable with secretion.

5. *Xylose is not reabsorbed.* It would appear probable that the normal tubular reabsorption of glucose, which prevails in all vertebrates that have been examined, is related to the metabolic importance of this substance and that the actual process of reabsorption possibly involves to some extent a process of metabolism, a suggestion which has been frequently made. In any case, one would not expect xylose, which is not metabolized to any appreciable extent by the rabbit or the dog (Corley, 1926, 1928; Greenwald, 1931) to be conserved by active tubular reabsorption in the manner of glucose. But if any active reabsorption occurred one would expect phlorizin, because it blocks the reabsorption of glucose, to block the reabsorption of xylose, also.

Xylose is excreted by all normal animals so far examined (glomerular fishes and the dog) with a relatively high U/P ratio when glucose is absent or present only in small quantities, in the urine. This fact in itself indicates that no extensive reabsorption of xylose prevails in the normal animal, but it is not sufficient to warrant the assumption that there is no reabsorption at all, because the quantitative relations between the plasma, the urine and the renal threshold might be different for the two sugars. So we have attempted to answer the question of whether xylose is reabsorbed by comparing the U/P ratios before and after phlorizin of glucose, xylose and a third and indifferent substance—indifferent, that is, so far as sugar metabolism is concerned. In the present experiments on the dog urea has been chosen for this third substance.

Discussion. With the background furnished by the above facts we may turn to the experimental data. The following experiments are given in full:

Table 1. Experiments 87, 88 and 100, showing the excretion of xylose, glucose and urea before and after the administration of phlorizin.

Table 2. Experiments 92 and 96, showing excretion of raffinose, glucose and urea before and after the administration of phlorizin.

TABLE 1

The effect of phlorizin on the excretion of xylose and glucose in the dog

EXPT. NUMBER	DURATION OF PERIOD	URINE VOLUME, V	UREA		XYLOSE		GLUCOSE		CM. = $\frac{UV}{P}$/S.A.			CM. GLUCOSE / CM. XYLOSE	CM. UREA / CM. XYLOSE
			Plasma	Urine	Plasma	Urine	Plasma	Urine	Urea	Xylose	Glucose		
	minutes	*cc.*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*					
87	26	0.885	10.4	271.0	99.1	6,260	121.8	1,090	29.9	72.7	10.3	0.14	0.41
	32	0.906	10.8	225.0	113.5	6,520	125.2	830	24.5	67.6	7.8	0.12	0.36
	31	1.29	11.2	134.5	112.8	3,970	115.8	4,067	20.1	59.0	58.9	1.00	0.34
	28	1.39	11.9	119.0	102.6	3,760	109.0	4,320	18.1	66.2	71.6	1.08	0.27
88	31	0.900			110.0	6,780	85.1	Trace		102.6*	0.0	0.0	
	30	1.066			114.0	5,940	85.0	Trace		102.9	0.0	0.0	
	32	1.312			89.0	3,940	75.7	3,260		107.6	104.6	0.97	
	31	1.290			96.0	4,270	71.2	2,930		106.3	98.4	0.93	
100	24	2.290	6.5	70.7	150.4	2,792			32.8	56.1			0.585
	26	1.537	6.5	98.9	152.7	4,040			30.8	53.6			0.575
	29	1.620	6.5	92.5	169.9	4,185	79.1	1,895	30.1	52.7	51.2	0.972	0.581
Average after phlorizin												0.99	

* Xylose clearance abnormally high, possibly because room was cold and dog shivered throughout experiment.

Expt. 87. Dog 18, wgt. 20 kgm., S.A. 0.77 sq. m. Mixed diet. Twenty-four grams xylose in 60 cc. water injected subcutaneously at 6:00 a.m. and 6 grams in 20 cc. water at 6:45 a.m. Water *ad lib.* Period 1 began at 7:03 a.m.

Two hundred milligrams phlorizin per kgm. in 50 cc. 1.25 per cent $NaHCO_3$ solution injected subcutaneously at end of period 2, or 8:02 a.m. Six grams xylose in 20 cc. water injected subcutaneouly at 8:45 a.m. Period 3 began at 9 a.m. All blood samples drawn at middle of urine collection periods.

Expt. 88. Dog 31, wgt. = 12 kgm., S.A. = 0.54 sq. m. Cracker meal diet. Fourteen and five-tenths grams xylose in 40 cc. water injected subcutaneously at 6:45 a.m. and 3.6 grams in 15 cc. water at 7:30 a.m. Three hundred cubic centimeters water by stomach at 6:50 a.m. and 300 cc. water at 7:30 a.m. Period 1 began at 7:45 a.m.

One hundred milligrams phlorizin per kgm. in 2.5 per cent $NaHCO_3$ injected intravenously and same quantity subcutaneously at end of period 2, or 8:45 a.m. Six grams xylose in 15 cc. water injected subcutaneously at 9:30 a.m. Period 3 began at 9:45 a.m. Blood samples drawn at middle of urine collection periods.

Expt. 100. Dog 20, wgt. 19.0 kgm., S.A. 0.76 sq. m. Cracker meal diet 3 days. Fasted 2 days; 46 grams xylose in 100 cc. water injected subcutaneously at 9:50 a.m. and 11 grams xylose at 10:35 a.m. Period 1 started at 10:56 a.m.

One hundred milligrams phlorizin per kgm. in $NaHCO_3$ intravenously and same quantity subcutaneously at end of period 2, or 11:55 a.m. Period 3 started at 12:40 a.m. Blood samples drawn at irregular intervals and interpolated.

Table 3. Experiments 84, 85 and 86, showing the excretion of xylose, sucrose and urea in the normal animal.

The facts to be noted in these experiments are as follows:

1. Xylose, sucrose and raffinose are normally excreted by the dog with a relative high U/P ratio, a fact which qualitatively indicates that these sugars are not actively reabsorbed by the tubules.

TABLE 2

The effect of phlorizin on the excretion of raffinose and glucose in the dog

EXPT. NUMBER	DURATION OF PERIOD	URINE VOLUME, V	UREA		RAFFINOSE		GLUCOSE		CM. = $\frac{UV}{P}$/S.A.			$\frac{\text{CM. GLUCOSE}}{\text{CM. RAFFINOSE}}$	$\frac{\text{CM. UREA}}{\text{CM. RAFFINOSE}}$
			Plasma	Urine	Plasma	Urine	Plasma	Urine	Urea	Xylose	Glucose		
	minutes	cc.	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*					
92	32	1.940	10.0	197.0	120.6	3,553	87.7	00.0	50.2	75.2	00.0	0.00	0.67
	29	1.380	9.7	262.0	107.4	4,900	84.8	00.0	49.0	82.9	00.0	0.00	0.59
	30	0.933	8.7	303.5	82.0	4,750	79.3	5,010	42.8	71.1	77.6	1.09	0.60
	31	0.967	9.1	276.0	70.9	4,324	78.8	5,075	38.6	77.6	81.9	1.05	0.50
96	30	2.233	6.5	86.4	90.2	2,551	75.5	00.0	39.6	84.4	00.0	0.00	0.47
	33	1.545	6.3	112.3	96.1	3,942	80.3	00.0	36.7	84.5	00.0	0.00	0.43
	49	0.725	6.8	208.3	119.9	5,710	73.7	3,590	29.6	46.0	47.1	1.02	0.64
	34	1.088	6.7	219.9	87.5	5,327	56.5	3,513	47.6	88.4	90.2	1.02	0.54
Average after phlorizin												1.04	

Expt. 92. Dog 20, wgt. 19 kgm., S. A. 0.76 sq.m. Cracker meal diet six weeks. Twenty-four grams raffinose in 100 cc. water injected subcutaneously and 300 cc. water by stomach at 6:10 a.m. Six grams raffinose in 30 cc. water subcutaneously at 7:00 a.m. Period 1 began at 7:14 a.m.

One hundred milligrams phlorizin per kgm. in $NaHCO_3$ solution subcutaneously and same quantity intravenously at end of period 2, or 8:10 a.m. Period 3 began at 9:17 a.m. All blood samples drawn at middle of collection periods.

Expt. 96. Dog 29, wgt. 13.5 kgm., S.A. 0.75 sq. m. Cracker meal diet four weeks. Twenty-four grams raffinose in 100 cc. water injected subcutaneously and 500 cc. water by stomach at 9:00 a.m. Six grams raffinose in 30 cc. water subcutaneously at 9:45 a.m. Period 1 began at 8:00 a.m.

One hundred milligrams phlorizin per kgm. in $NaHCO_3$ solution intravenously and 200 mgm. subcutaneously at end of period 2, or 9:10 a.m. Fourteen grams raffinose in 60 cc. water subcutaneously and 500 cc. water by stomach at 11:40 a.m. Period 3 began at 12:10 p.m. All blood samples drawn at middle of collection periods.

2. Phlorizin does not cause a rise in the absolute value of the U/P ratio for these sugars, as it does in the case of glucose, nor does it cause a rise in clearance (i.e., the quantity of sugar excreted per unit time from a constant blood level, or UV/P) as would be expected if active reabsorption normally occurred and if this reabsorption were blocked by phlorizin.

3. The ratio of the xylose or raffinose clearance to the urea clearance is not significantly affected by phlorizin; there is no reason to believe, even if this drug blocked the reabsorption of these sugars, that it would block the reabsorption of urea to exactly the same extent, if at all; therefore, since the sugar clearance is not increased relative to the urea clearance after

TABLE 3

The excretion of urea, xylose and sucrose in the normal dog

EXPT. NUMBER	DURATION OF PERIOD	URINE VOLUME, V	UREA		XYLOSE		SUCROSE		$CM. = \frac{UV}{P}/S.A.$			CM. SUCROSE / CM. XYLOSE
			Plasma	Urine	Plasma	Urine	Plasma	Urine	Urea	Xylose	Sucrose	
	minutes	*cc.*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*				
84	32	5.72	15.5	110.8	57.1	488	64.5	555	53.8	64.4	64.8	1.01
	31	6.22	15.3	108.0	78.3	571	94.6	688	57.8	59.6	59.6	1.00
	33	4.27	15.2	145.5	69.0	816	75.0	915	53.8	66.5	68.6	1.03
85	32	1.08	12.5	165.3	98.1	1,652	109.2	1,880	26.4	33.7	34.4	1.02
	28	1.09	12.9	146.5	106.4	2,019	102.4	2,116	22.9	38.3	41.7	1.09
	30	1.03	13.5	175.0	116.4	2,086	115.2	2,308	24.7	34.2	38.2	1.12
86	31	1.03	10.5	257.0	95.8	4,025	104.8	4,075	33.2	57.0	52.7	0.92
	29	1.17	11.0	215.0	105.2	4,130	108.8	4,398	30.1	60.6	62.2	1.03
	32	1:22	9.5	203.0	101.3	3,820	109.1	4,210	34.3	60.6	62.0	1.02
Average												1.01

Expt. 84. Dog 20, wgt. 19.4 kgm., S.A. 0.76 sq. m. Cracker meal diet 11 days. Fifteen grams each xylose and sucrose in 60 cc. water injected subcutaneously at 6:00 a.m., and again at 8:00 a.m. Seven hundred fifty cubic centimeters water by stomach at 5:55 a.m. and 500 cc. at 7:55 a.m. Period 1 began at 8:08 a.m. All blood samples drawn at middle of urine collection periods.

Expt. 85. Dog 31, wgt. 12 kgm., S.A. 0.54 sq. m. Cracker meal diet 22 days. Nine grams each xylose and sucrose in 36 cc. water injected subcutaneously at 6:15 a.m. and again at 7:00 a.m. Four hundred cubic centimeters water by stomach at 6:20 a.m. and 200 cc. at 7:05 a.m. Period 1 began at 7:15 a.m. All blood samples drawn at middle of urine collection periods.

Expt. 86. Dog 20, wgt. 19.0 kgm., S.A. 0.76 sq. m. Cracker meal diet 31 days. Thirty-two grams xylose and 24 grams in 100 cc. water injected subcutaneously at 6:05 a.m. and 8 gms. xylose and 6 grams sucrose in 20 cc. water at 6:50 a.m. No water given. Period 1 began at 7:06 a.m. All blood samples drawn at middle of urine collection periods.

phlorizin, it may be inferred that phlorizin is without effect upon the former.

4. Under the influence of phlorizin the glucose clearance rises to the xylose clearance or the raffinose clearance, as the case may be, but never significantly exceeds the latter. This indicates that there is no active reabsorption of xylose or raffinose which is *not affected* by phlorizin; for if

such reabsorption existed then the glucose clearance under phlorizin should rise above the xylose or raffinose clearance. This conclusion would be invalid if the action of phlorizin in blocking the reabsorption of glucose were an incomplete one, but all the evidence available on this subject indicates that in sufficient doses the contrary is the case; in fact, it has been observed in this laboratory that as little as 25 mgm. of phlorizin per kilogram given intravenously suffices to bring the glucose clearance up to that of xylose for short periods of time (1 to 2 hours), and larger doses do not raise it any higher. In experiments 88, 92, 96 and 100, we have given four times this quantity intravenously and an equal quantity subcutaneously, so we believe that the reabsorption of glucose is completely blocked.

We conclude from the above facts that there is no active reabsorption of xylose or raffinose in the kidney of the normal dog.[1]

It remains possible, however, that a small quantity of sugar might be reabsorbed into the blood stream by passive diffusion from the lumen of the tubule, since during the reabsorption of water a large concentration gradient is established between the urine and the plasma. That such a passive diffusion must be slight is indicated by the fact that urea can be concentrated 250 times, and urea is more diffusible than any of the sugars; but since it is possible that urea is itself extensively reabsorbed, this evidence is hardly conclusive. Therefore, to examine this question further we have compared the excretion of xylose with sucrose in the normal dog. Sucrose is not excreted by the tubules of *Lophius*, as shown by Clarke and Smith (1932), in any detectable quantity; certainly less than one per cent diffuses across the renal tubules from the plasma into the urine. (The excretion of raffinose by the aglomerular kidney has not been examined.) The molecular weights of unhydrated xylose, sucrose and raffinose are 150, 342 and 504, respectively, and it is to be expected that their rates of diffusion would vary inversely as the molecular size.

5. The data given in table 3 show that xylose and sucrose are excreted by the normal kidney in an identical manner within the limits of the ex-

[1] It seems hardly necessary to discuss the view that phlorizin induces the secretion of glucose by the renal tubules. The extensive studies which have been made on the *modus operandi* of this drug have demonstrated to the satisfaction of most investigators that it acts by paralyzing the tubular reabsorption of glucose from the glomerular filtrate (Nash, 1927). There is conclusive evidence in Marshall's (1930) work on the fishes that the renal tubules are unable to secrete glucose and this fact, even if there were no other evidence, would lead us to believe that the tubular secretion of glucose in the mammals is a fiction. But we now have the additional evidence in our subjoined tables to the effect that the physiologically inert sugars, xylose, sucrose and raffinose, when excreted simultaneously are excreted in an identical manner (i.e., with an identical clearance) in the normal animal; a principle which is extended to glucose in the phlorizinized animal. These observations leave no doubt whatsoever that the excretion of all these sugars is effected exclusively by glomerular filtration.

perimental error in the determination of the sugars. The data given in tables 1 and 2 show that under phlorizin, glucose and xylose, on the one hand, and glucose and raffinose, on the other, are likewise excreted in an identical manner; so that except for the use of phlorizin to block the reabsorption of glucose, we may say that all these sugars are handled simultaneously by the kidney in exactly the same way. It would seem, therefore, that they must be reabsorbed to exactly the same extent, or else not reabsorbed at all. Since we are comparing substances of different molecular weights and diffusibility, the first alternative is very unlikely. This conclusion is reinforced by the observations of Clarke and Smith (1932) that the tubules of *Lophius* do not permit xylose and sucrose to diffuse across them, whereas urea is nearly uniformly distributed between the blood and urine (Marshall and Grafflin, 1928). In the dog, on the other hand, where urea may be concentrated 250-fold it is inconceivable that the tubules would permit the passive diffusion of appreciable quantities of substances like raffinose, sucrose, xylose and glucose.

In résumé, the facts that the xylose clearance relative to the urea clearance is unaffected by phlorizin, that the glucose clearance under phlorizin does not exceed the xylose clearance or the raffinose clearance, and that the xylose and the sucrose clearances in the normal animal and the glucose and xylose or glucose and raffinose clearances in the phlorizinized animal are identical (in simultaneous experiments), are interpreted to indicate that neither xylose, sucrose nor raffinose is reabsorbed by the renal tubules, and therefore, in vew of the arguments set forth above, any of these substances may be used to measure the glomerular filtrate with a theoretical accuracy lying within the limits of the experimental error in the sugar analysis.

Of the three sugars examined xylose and sucrose are the most suitable for practical purposes. The accurate determination of xylose is perhaps not so easy as is the determination of sucrose, but the former possesses the advantages that it can be administered *per os* and its chemical determination is not dependent upon the use of an enzyme.

The question of whether the administration of these sugars influences the excretory activity of the kidney with respect to other substances cannot be answered in this paper. It would seem that an inert sugar would exert as little action as any substance which might be used for the measurement of the glomerular clearance. But it is recognized that many factors, only a few of which are known at present, modify the activity of the kidney, and although we believe that these sugars have no specific stimulating or depressing action, either in respect to the excretion of urea or any other substance, the examination of this point must be postponed until certain conditions bearing on the maintenance of uniform renal activity can be discussed.

SUMMARY

It is shown that xylose is excreted by the normal dog kidney, at moderate to large urine flows, with a relatively high U/P ratio; under the action of phlorizin the glucose clearance rises to but never exceeds the xylose clearance, while the xylose clearance itself, or the xylose clearance relative to the urea clearance, remains unaffected. These facts indicate that there is no active reabsorption of xylose by the renal tubules.

The pentose, xylose, the disaccharide, sucrose, and the trisaccharide, raffinose, all of which are physiologically inert, are excreted (in simultaneous experiments) in an identical quantitative manner relative to each other or to glucose under phlorizin, indicating that there is no significant passive reabsorption (by diffusion) from the concentrated urine in the tubules.

From these and other considerations described in the paper we believe that these sugars can be used to evaluate the quantity of glomerular filtrate with an error that does not exceed a few per cent.

Methods for the analysis of mixtures of xylose, glucose and sucrose, or of glucose and raffinose in plasma and urine are described.

The xylose used in these experiments was kindly supplied by the Swann Chemical Company of Birmingham, Alabama. We are indebted to Miss Annie Breitweiser for her expert technical services in sugar analysis.

METHODS. A careful comparison of several sugar methods, tested by the recovery of added amounts of various sugars to plasma and urine, led us to choose the Folin (1929) method as yielding the best results in our hands. The Folin sugar reagent possesses the advantage that it is not reduced by phlorizin, which substance reduces ferricyanide; this fact, in our opinion, renders suspect all quantitative glucose observations on phlorizinized animals made with a ferricyanide method. The preparation of reagents and the sugar analysis were carried out precisely as described by Folin except that we have used copper filtrates as prepared by Somogyi (1931). Special blow-out pipettes were used in all measurements and great care was exercised in all details of technique.

Glucose + xylose. A portion of serum or oxalated plasma is added to 7 volumes of water in a 125 cc. Erlenmeyer flask; one volume of copper solution and one volume of sodium tungstate solution are added while spinning and the precipitated proteins are filtered out after 15 minutes using S. and S. 597 filter paper. Two cubic centimeters of this filtrate are taken for sugar analysis according to Folin (1929). Urine is diluted according to the expected sugar content, usually 1:25 or 1:50, with 0.06 per cent benzoic acid. A copper filtrate is prepared from this diluted urine as above. Glucose, xylose and sucrose will remain unchanged in the diluted urine over-night if kept in the ice-box.

Xylose. A weighed sample of yeast is made up to a 20 per cent suspension in water and washed by centrifuging until the supernatant fluid is clear (usually four times); 5 cc. of this suspension are centrifuged in a 15 cc. centrifuge tube for 15 minutes at high speed, decanted and drained for 30 minutes, when the sides of the tube are wiped dry up to the edge of the yeast. Five cubic centimeters of plasma or urine filtrate are added to the 1 cc. of dry yeast, stirred well and allowed to stand at room

temperature for 15 minutes with occasional agitation. The mixture is then centrifuged for 15 minutes to throw down the yeast and 2 cc. of the supernatant fluid are used for sugar analysis. (After Somogyi, 1928, and Van Slyke and Hawkins, 1929.)

We find that xylose is not actively absorbed by yeast, but that a small quantity (depending upon the proportion of yeast and filtrate) disappears each time during successive yeast treatments. This fraction is about 13 per cent when the proportions of 1 cc. of dry yeast to 5 cc. of fluid are used. This absorption probably represents a moiety which diffuses into the yeast. (Cf. also Van Slyke and Hawkins, 1929, who found partial absorption of non-fermentable substances.) In this respect we do not confirm Raymond and Blanco (1928) who obtained complete recovery of xylose after yeast treatment and who make no mention of passive diffusion. The reducing power of pure xylose by the Folin sugar method in our hands is 85 per cent of that of glucose in contrast to 78 per cent obtained by Poe and Klemme (1930); consequently after yeast treatment as above we recover 72 per cent of added xylose. This low recovery is theoretically and actually constant and can be neglected in the calculation of U/P ratios when the plasma and urine filtrates are treated in exactly the same manner. Consequently we do not make any correction in our data for reducing power of xylose, but present only the observed glucose equivalent.

In calculating the true glucose, however, allowance must be made for the diffusion of xylose into the yeast; therefore we calculate the glucose content of both plasma and urine as glucose + apparent xylose—apparent xylose/0.87.

Sucrose. The total sugar content of plasma and urine filtrates prepared as above, and containing glucose, xylose and sucrose was determined by inversion with sucrase. We are indebted to Mr. Milton Levy, of the Department of Biochemistry, for the sucrase used in these experiments. The enzyme was prepared from yeast by Hudson's method (cf. Morrow, 1927); we use a 0.26 per cent solution of a preparation 50 mgm. of which reduces the rotation of 20 cc. of 20 per cent sucrose solution to zero degrees in twelve minutes.

We find that sucrose is quantitatively absorbed by yeast, either the small tin-foil packages or baker's loaves, almost instantly. One cubic centimeter of yeast completely removes 40 mgm. from 5 cc. of solution (corresponding to 800 mgm. per cent in our method) in less than 5 minutes. In this observation we do not confirm Raymond and Blanco (1928) who observed only partial absorption, possibly because the yeast which they used was not fresh. (Cf. also Ronzoni and Somogyi, 1929.)

One cubic centimeter of plasma or urine filtrate is placed in a Folin sugar tube with one drop of 0.04 per cent bromocresol green, and 0.05 N acetic acid is added drop by drop until the color indicates a pH between 4.0 and 4.6. One cubic centimeter of sucrase is added and the mixture is agitated and allowed to stand in a water bath at 40°C. for 30 minutes or more after which it is neutralized with 0.05 N NaOH. One drop of phenolphthalein is added and the mixture is made alkaline with one per cent Na_2CO_3 as in Folin's sugar method. The standards are treated by adding the bromocresol green, acetic acid and NaOH. 2.0 cc. of Folin reagent are then added and the routine sugar analysis completed. Blanks must be made on the sucrase solution by adding it to known glucose solutions.

Sucrose is calculated as the difference between the glucose equivalents before and after inversion. The glucose equivalent of pure sucrose solutions treated as above is 105 per cent of glucose, and we obtain recoveries of added amounts (100 to 300 mgm. per cent) from plasma and urine with an error of ± 2 per cent. In the Folin method the reducing power of glucose is not impaired by the presence of sucrose in approximately equal concentrations, and since sucrose is completely removed by yeast (cf. above) it offers no interference to the determination of xylose.

Raffinose. Raffinose (a non-reducing trisaccharide) is hydrolyzed by sucrase to fructose and melibiose. Both sugars reduce Folin's Cu reagent, but the latter is

not absorbed on yeast. By the above method we find the reducing power of hydrolyzed raffinose at concentrations of 100 to 200 mgm. per cent to be 60 per cent of glucose. Since melibiose is not absorbed by yeast, it is not possible to determine xylose in the presence of raffinose by sucrase hydrolysis, so we have compared the excretion of raffinose with glucose under phlorizin, the analyses being made in the same manner as in the case of sucrose. Our data express glucose equivalents of raffinose as calculated from the difference between the glucose equivalents of solutions before and after hydrolysis.

When present in approximately equal concentrations raffinose does not interfere with the quantitative determination of glucose.

All plasma and urine filtrates are prepared in duplicate and these duplicates are analyzed separately with separate standards. All analyses in which the unknown differs from the standard by more than 20 per cent are repeated, using a more appropriate standard. No standard corresponding to more than 200 mgm. per cent or less than 50 mgm. per cent was used.

Urea. Urea in both plasma and urine was analyzed by Van Slyke's (1927) plasma method, the plasma and urine analyses being alternated to prevent poisoning of the urease in the extraction chamber. One or 2 cc. samples of plasma and 1 cc. of diluted urine were used. All analyses were run in duplicate for 3 and 6 minutes, the variation in time serving to check the activity of the urease. The agreement between checks, even at concentrations of 6 mgm. per cent is usually better than 2 per cent.

General. Except as noted the general technique is similar to that used by Jolliffe and Smith (1931a, b). The dogs were maintained upon a cracker-meal and butter or lard diet. The greatest care was exercised in catheterizing to insure complete removal of urine from the bladder. In the present experiments the sugars were injected subcutaneously some time before the first urine collection period; injection is not necessary in the case of xylose which may be given by stomach, but sucrose and raffinose must obviously be administered parenterally. The essential details of each experiment are recorded in the tables.

Note on the filterable nature of plasma glucose (cf. p. 302): After submitting this paper we noted the work of Powers and Greene (1931) showing that all the plasma glucose is filterable by *in vivo* dialysis.

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ACTION OF EPINEPHRINE ON THE INTESTINE FOLLOWING STIMULATION BY PARASYMPATHETIC DRUGS

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There have been occasional and sporadic reports in the literature of an anomalous action of epinephrine on the intestine; namely, the addition of this drug causes a contraction. Thus Salvioli (1902) injected epinephrine intravenously into a cat and observed after a small relaxation a marked contraction of the ileum. This was confirmed by Magnus (1903). In corroboration with this Bunch (1898) showed that electrical stimulation of the splanchnics may cause intestinal contractions. All these experiments were done with the intestine in situ and the nerve supply intact. One observation reported by Sharpey-Schäfer (1924) in his book shows an isolated piece of intestine contracted by the addition of epinephrine. Recently these observations have been confirmed in the case of ephedrine by Kreitmair (1927) and Kinnaman and Plant (1931) who show that large concentrations of ephedrine contract the intestine. They assume that ephedrine acts on the intrinsic motor mechanism (Auerbach's plexus) of the intestine, because in plexus free preparations no contraction occurs. Magnus observed this also in the case of epinephrine.

While working with isolated intestinal strips, we observed that the addition of epinephrine to intestine already partially contracted by pilocarpine always caused a greater contraction followed after some time by a slow relaxation. The rapidity with which relaxation set in varied with different preparations. Occasionally it immediately followed the rise, in other cases the rise was maintained for as long as a minute, and in still other cases no appreciable relaxation occurred. The rise, however, was a constant factor, the height of which depended on how far the intestine was already contracted by the pilocarpine. These results were also obtained when pilocarpine was substituted by physostigmine, or under certain conditions by histamine. In no case did we observe a contraction when epinephrine was added alone.

The interaction of pilocarpine and epinephrine was first worked out by Hirz (1913) who showed that these drugs worked antagonistically on the intestine of dogs and cats. Although his curves show no rise after the

addition of epinephrine, there is a distinct latent period before relaxation sets in. Recently Takeda (1930) has shown that epinephrine acts most powerfully as depressant on unstimulated rabbit's intestine, but after stimulation with pilocarpine or physostigmine the relaxation is almost abolished.

EXPERIMENTAL. The Burn and Dale apparatus was used. The experiments were performed on the intestine of guinea pigs which were starved 24 hours and lightly anesthetized with ether before decapitation and removal of the ileum. Pieces 2 cm. long were tied off with an empty lumen and suspended in the bath at 37°C. A modified Ringer solution without phosphate carbonate or glucose was used aerated with carbon dioxide free air. The intestine was suspended in 45 cc. of this solution.

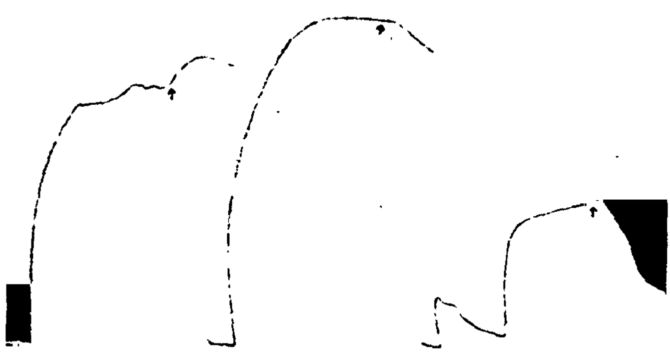

Fig. 1. First curve: contraction with 0.2 cc. pilocarpine followed by 0.2 cc. epinephrine added at arrow. Second curve: Contraction with 0.2 cc. histamine, 0.2 cc. epinephrine added at arrow. Third curve: The first contraction due to 0.01 cc. nicotine, the second contraction to 0.2 cc. histamine. 0.2 cc. epinephrine added at arrow. The three curves obtained on the same piece of ileum. The different height of contraction is not a factor. Similar results are obtained when the response to histamine and pilocarpine is the same.

The drugs were made up in the following concentrations: pilocarpine sulfate (Merck) 5 mgm. in 25 cc.; physostigmine hydrochloride (Merck) 10 mgm. in 25 cc.; histamine acid phosphate (British Drug Houses) 2.5 mgm. in 25 cc.; and adrenalin (Parke, Davis) 1 part in 1000. Both the adrenalin tablets and the solution containing chloretone were used with similar results.

The first curve in figure 1 shows the effect of 0.2 cc. of epinephrine added after contraction by 0.2 cc. of pilocarpine. In this curve the intestine shows no peristalsis, but in preparations where there is active peristalsis the same phenomenon occurs. If physostigmine is substituted for pilocarpine, epinephrine has the same effect. Variations in the amount of pilocarpine added up to 1 cc. did not affect the result. Nor did the concentration of epinephrine even when as much as 2 cc. were added

affect the shape of the curve. If the intestine was contracted to its fullest extent, the rise after epinephrine would be very slight and occasionally absent. To avoid this only small amounts of pilocarpine were added.

When histamine is substituted for pilocarpine, the addition of epinephrine very seldom causes a contraction, but there is a distinct latent period before the relaxation occurs. Should a contraction occur, it is always immediately followed by a relaxation and the contraction is inconspicuous compared to the one caused by epinephrine in the presence of pilocarpine. Histamine acts primarily directly on smooth muscle but it also has some action on the parasympathetics, for atropine will cause a relaxation after histamine as shown by McKaye (1930) and Bernheim (1931). Nicotine will inhibit the contraction by histamine to about the same extent as atropine. In the presence of nicotine or atropine the latent period seen on the addition of epinephrine after histamine alone is considerably shortened and occasionally completely abolished and the extent of the relaxation is much greater. This is shown in curves 2 and 3 in figure 1. The atropine curve is similar to the nicotine curve shown here. The three curves in figure 1 were obtained on the same strip of intestine which was thoroughly washed between the additions of the drugs. When the nicotine is washed away, a curve similar to the second curve is obtained.

The duodenum behaves similarly to the ileum in all these respects, but the action of epinephrine is not as clear cut or as regular as on the ileum.

Discussion. It is obvious from these experiments that parasympathetic stimulation is necessary before a contraction by epinephrine is possible. Thus after pilocarpine or physostigmine contractions by epinephrine are regularly seen whereas after histamine contractions are rarely seen, but there is a definite latent period before relaxation. This corresponds to the fact that histamine stimulates the parasympathetic very slightly compared to pilocarpine and physostigmine; moreover, parasympathetic poison such as atropine and nicotine inhibit to a certain extent the histamine contraction, cut down the latent period, and increase the relaxation by epinephrine. As regards the older observations on the contraction of the intestine by epinephrine, it is significant that with the exception of a single observation by Sharpey-Schäfer, they were made on the intestine in situ with the nerves intact, where presumably vagus tone if not actual stimulation was in effect.

Epinephrine, a sympathetic stimulant, acts further to contract guinea pig's intestine already partially contracted by parasympathetic stimulation. This fact, with the observation of Carlson, Smith and Gibbons (1927) that acetylcholine may contract or relax cat's intestine depending upon its state, the observation of Bernheim (1931) that pilocarpine may relax a contracted duodenum of the guinea pig, plus the additional evidence that electrical stimulation of the vagus may cause intestinal relaxation

in the cat (Melik-Megrabow, 1926), in the turtle (Rogers and Bercovitz, 1921) in the dog (Bercovitz and Rogers, 1921) and that electrical stimulation of the splanchnics may cause intestinal contraction in the dog (Bunch), indicates that the conception of a rigid antagonism between the sympathetics and parasympathetics in the intestine is no longer strictly valid. Instead the reaction of one of these systems is not immutable, but is a function of the reactivity of the other or the state of the intestine itself. Thus under one set of conditions they may be antagonistic, under another they may reinforce one another, and under a third their usual rôles may be reversed.

It might be well to add here that, due to the difference in the action of epinephrine after pilocarpine and after histamine, the contraction caused after the former cannot be due to impurities in the commercial preparation but must be due to the epinephrine itself. Also we have occasionally had intestines which reacted very slowly to pilocarpine. In these cases addition of epinephrine causes a contraction much greater than those recorded above but because the pilocarpine action was not typical we have not included a record of them here.

SUMMARY

1. Epinephrine added to the ileum of a guinea pig which has been contracted by pilocarpine or physostigmine causes a further contraction which may or may not be followed by relaxation.

2. After histamine, epinephrine always causes relaxation, but the relaxation occurs after a definite latent period. The relaxation is more rapid and the latent period shorter in the presence of nicotine or atropine.

3. These facts indicate that epinephrine may act as an adjuvant to parasympathetic drugs and that its action is dependent on the amount of parasympathetic stimulation.

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STUDIES OF GALL-BLADDER FUNCTION

VII. The Anion-Cation Content of Hepatic and Gall-Bladder Bile[1]

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We have previously reported the fate of various concentrations of sodium chloride, calcium lactate, bile salts, cholesterol and a mixture of bile salts and cholesterol when placed separately in the bile-free gall bladder of the unanesthetized dog (1932a, b, c). The method used in those studies permitted us to study fluids quantitatively in a gall bladder which was bile-free, sterile and at the same time retained its blood supply and major lymphatic vessels. Furthermore, hyperbilirubinemia did not occur and the feces were always normal in color.

It seemed highly desirable to use the same method in order to study the changes in the anion-cation concentration of hepatic bile when this was subjected to gall-bladder activity. We are not aware of any studies of anion-cation balance of hepatic and gall-bladder bile. Gamble and McIver (1928) published data on the total base and chloride of the hepatic and gall-bladder bile of the cat. They did not study the bicarbonate or bile acid concentration, nor were their studies quantitative in that they did not subject known amounts of hepatic bile, or measured concentrations, to gall-bladder activity. From the studies which they made definite conclusions cannot be drawn as to whether the observed differences in the bile were due to absorption, to secretion or excretion, or to a combination of these processes.

Method. The hepatic bile was obtained from cholecystectomized dogs, following double catheterization of the common duct. By this method we were able to collect bile at will or to shunt it into the distal portion of the duodenum. The proximal catheter was inserted to a point just below the insertion of the right and left lobe ducts. The distal catheter was introduced to the point where the common duct began to traverse the

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duodenal wall. If the catheter was inserted into the duodenal lumen infection was much more apt to occur.

The bile was collected for twenty-four hours and then the flow was turned into the distal catheter Thus the animals were kept healthy and the appetite did not suffer materially. The operations as well as all collections were done under strictly aseptic precautions. Cultures or smears were made frequently and the animals were discarded when evidence of infection was obtained.

The dogs used for the study of gall-bladder activity were prepared by the same method which we used in our previous studies (1932a). The same care was taken in these animals as was taken with the fistula animals to prevent infection.

Only data from animals whose gall bladders were proven to be sterile are being reported in this paper. The activity of the damaged gall bladder is so different from the normal that we are reporting the observations on the damaged gall bladder separately.

The gall bladder of every animal used for the activity experiments was first filled with a solution of sodium chloride (152 m.Eq. per liter) after carefully cleansing it with this solution. Every preparation was tested at operation so as to be sure that we could recover the exact amount of fluid introduced. Animals with unligated accessory ducts were discarded, as were animals where the rate of absorption of the sodium chloride was not comparable to that which we had observed in the earlier experiments (1932a). The experiments were begun after recovery from the anesthetic.

Hepatic bile in known amounts was introduced into the bile-free gall bladder and permitted to remain there for from two to twenty-four hours. It was removed with a Luer syringe. As long as the gall bladder remained normal fluid was absorbed. The exact amount varied considerably, the maximum being 6.75 cc. per hour, the rate decreasing as the bile became concentrated. Estimations of the pigment concentrations made by us in hepatic bile subjected to gall-bladder activity have shown increases in concentration of this substance over a 24 hour period varying from 5 to 17 times that of the bile introduced.

The methods used in our analyses were substantially the same as those reported in our earlier papers (1932a, b, c).

The pH determinations were done with the glass electrode of Stadie, O'Brien and Laug (1931).

The freezing point determinations were done by Sunderman using the method of Stadie and Sunderman (1931).

Results. *Hepatic bile.* This bile is more variable in its electrolyte composition than is serum. This is true for hepatic bile from different animals and for the same animal at different times. The variation occurs chiefly in the ratio between chloride, bile salt and bicarbonate (table 1).

We have made no attempt to study the total 24 hour anion-cation output so that we cannot state whether taken for such a period the anion-cation content of hepatic bile in the same animal is constant under similar conditions. The total base concentrations of hepatic bile have varied from 174 to 192 m.Eq. per liter.

On the whole, hepatic bile is higher in base, total anion and as a rule in bicarbonate than is serum. The chloride concentration of hepatic bile tends to be somewhat lower than that of serum. The bile salt concentration has varied from 15.6 to 66 m.Eq. per liter. There is considerable variation in the calcium concentration of the hepatic bile at different periods in the same dog and in different dogs. We have obtained concentrations varying from 3.1 to 9.9 m. Eq. per liter. In the majority of instances the concentration is higher than that of serum. The pH has varied from 7.1 to 8.6. As a rule it is above that of serum.

TABLE 1

Hepatic bile data

DOG NO.	BASE	CALCIUM	CHLORIDE	CO_2	BILE SALT
	m.Eq./L.	*m.Eq./L.*	*m.Eq./L.*	*m.Eq./L.*	*m.Eq./L.*
535		3.4	55.0	64.8	42.0
96	181	6.3	103.9	34.4	29.6
96	179	5.8	89.6	45.1	28.1
96	183	6.5	63.7	62.8	30.0
91	188	5.9	62.1	58.9	42.1
91	192	9.9	93.2	34.7	45.9
188	189	6.9	89.2	45.1	32.9
188	174	7.0	93.6	40.6	36.7
188	176	6.9	107.0	45.8	34.2

The amount of anion which might be accounted for by the small amount of protein present in hepatic bile of the dog is not more than a few milli-equivalents per liter. A comparison of the determined anion-cation concentrations shows that unknown anions account for a median of about 12 milli-equivalents per liter. The amount of cation bound by phosphoric acid either inorganic or in lipoid combination is unknown. No attempt was made to determine the concentration of fatty acids or ethereal sulphates in hepatic bile.

Gall-bladder bile. After subjecting a single injection of hepatic bile to gall-bladder activity for a short period (average 5 hours) there was considerable variability in the composition of the bile. In seven of the nine short experiments where complete studies were made the per cent decrease of total determined anion was greater than that of cation. In the remaining two experiments total determined anion showed the same per cent decrease as cation.

There is apparently no correlation between the exact time intervals and the changes in the anion concentration which occurred during the short period. The undetermined anion concentration increased in the gall-bladder bile to a median of 26 m.Eq. per liter. Whatever this may be we

TABLE 2
Anion-cation studies
Time average 5 hours

DOG NO.	HEPATIC BILE						GALL-BLADDER BILE					
	Total base	Measured anion	Chloride	Bile salt	$BHCO_3$	pH	Total base	Measured anion	Chloride	Bile salt	$BHCO_3$	pH
	m.Eq./L.	*m.Eq./L.*	*m.Eq./L.*	*m.Eq./L.*	*m.Eq./L.*		*m.Eq./L.*	*m.Eq./L.*	*m.Eq./L.*	*m.Eq./L.*	*m.Eq./L.*	
122 (1)	184.0		94.3		22.2	7.9	173.0		70.3		14.8	6.7
(3)	177.0		94.5	24.1			167.0		50.4	43.0		
184 (2)	181.0	167.9	103.9	29.6	34.4	7.2	178.0	162.4	91.6	65.2	5.6	6.3
(3)	179.0	162.8	89.6	28.1	45.1	7.1	157.0	142.1	102.0	34.4	5.7	6.4
(4)	177.0	173.1	84.5	61.0	27.6	7.5	154.0	134.8	99.2	31.4	4.2	6.5
(5)	183.0	156.5	63.7	32.0	62.8	8.1	169.0	132.0	77.2	24.3	30.5	7.0
(7)	188.0	163.1	62.1	42.1	58.9	7.4	178.0	152.5	86.8	45.7	20.0	6.7
190 (1)	192.0	173.8	93.2	45.9	34.7	7.9	210.0	169.5	70.6	77.8	21.1	7.2
(3)	189.0	167.2	89.2	32.9	45.1	7.9	198.0	133.5	27.6	61.6	44.3	7.5
(4)	174.0	170.9	93.6	36.7	40.6	7.8	167.0	139.7	47.8	34.4	57.5	7.4
(5)	176.0	187.0	107.0	34.2	45.8	8.2	151.0	141.6	70.2	37.8	33.6	7.4

TABLE 3
Summary of data of short experiments

	NUMBER OF EXPERIMENTS	INCREASE IN CONCENTRATION	DECREASE IN CONCENTRATION	HEPATIC BILE		GALL-BLADDER BILE	
				Maximum	Minimum	Maximum	Minimum
				m.Eq./L.	*m.Eq./L.*	*m.Eq./L.*	*m.Eq./L.*
Base	11	2	9	192.0	174.0	210.0	151.0
Calcium	20	20	0	9.7	5.3	25.7	6.9
pH	17	0	17	8.6	7.1	7.5	6.3
$H\bar{C}O_3$	15	1	14	62.8	22.2	57.5	3.2
Free CO_2	10	9	1	4.5	0.4	5.9	1.1
Chloride	31	5	26	107.0	62.1	102.0	1.9
Bile salt	27	22	5	65.7	15.6	194.2	24.3
X anion	9	8	1	26.9	0.0	62.4	7.7

have called it the "x" anion. It increases in concentration when hepatic bile was subjected to gall-bladder activity.

In table 2 are included sufficient data to show the types of variation which we have observed in these short experiments and in table 3 a sum-

mary of all the short experiments. In the latter complete data are at times lacking. Total base as a rule decreased in concentration while the calcium concentration increased consistently, and bile salt concentration increased in 82 per cent of the experiments. Bicarbonate concentration decreased, and in 84 per cent of the experiments the same was true for chloride. The free CO_2 increased and the pH of hepatic bile decreased when subjected to gall-bladder activity.

In the two experiments where total base concentration increased the sum of the bile salt and undetermined anion concentrations increased to above

TABLE 4

Hepatic bile subjected to gall-bladder activity—additive experiments

DOG NO.	DATE		HEPATIC BILE INTRODUCED	RECOVERED FROM GALL BLADDER	REMOVED FOR ANALYSIS	TOTAL BASE	CALCIUM	CHLORIDE	CO_2	BILE SALT
			cc.	*cc.*	*cc.*	*m.Eq./L.*	*m.Eq./L.*	*m.Eq./L.*	*m.Eq./L.*	*m.Eq./L.*
453	11/13/31	Bile removed*				284	25.6	4.1		239
	11/13/31		28.0	2.5	0.0					
	11/14/31		31.0		4.0		26.3	5.6		114
	11/15/31		31.0	5.0	5.0	224		7.3		227
410	11/17/31	Bile removed*					23.4	10.3		189
	11/17/31		20.0	1.8	0.0					
	11/18/31		37.0	7.0	7.0	241	26.4	8.2		282
490	11/23/31	Bile removed*				305	30.9	1.1	0.9	334
	11/23/31		44.0	3.5	0.0					
	11/23/31		17.0							
	11/24/31		24.0	4.5†	0.0					
	11/24/31		7.0		7.0‡	235		33.7	16.9	229
	11/24/31				7.0	240	31.1	2.1	6.7	252

* Gall-bladder bile removed at time of operation.
† Recovered before addition of 24.0 cc.
‡ Removed before addition of 7.0 cc.

45 per cent of the total base concentration. In the remaining seven experiments where total base decreased, the sum of the bile salt and undetermined anion concentrations was less than 45 per cent of the total base concentration. There appears to be an approximately linear relationship between changes in total base and the ratio of the bile salt plus undetermined anion concentration to the total base; as the bile salt plus undetermined anion comes to balance a larger proportion of the total base, decrease in total base concentration gives way to increase.

Since bile salt and undetermined anion tended to increase in concentration we felt it advisable to conduct experiments in which we could expect

maximum concentrating effects and still have sufficient material for analysis. After preparation as previously described known amounts of hepatic bile were added at intervals to the gall bladder. Specimens just sufficient for analysis were removed at intervals and further additions of hepatic bile continued. These experiments were carried over a period of from 24 to 48 hours. When this was done the results were different than those obtained in the short experiments (table 4).

In these experiments chloride and bicarbonate concentrations were reduced while calcium, bile salt and total base concentrations were constantly increased as long as the gall bladder remained normal. In each instance the distribution of the electrolytes was similar to that found in the gall bladder bile removed at operation. In one instance the activity was such that the bile removed after a period of study was greater in calcium and bile salt and less in chloride concentration than that originally removed from the same gall bladder. In every instance the equivalent

TABLE 5

Depression of the freezing point

DOG NO.	BASE	BILE SALT	CALCIUM	CHLORIDE	CO_2	CHOLESTEROL	Δ
				Hepatic bile			
	m.Eq./L.	m.Eq./L.	m.Eq./L.	m.Eq./L.	m.Eq./L.	mgm./100 cc.	
535	174	42.0	3.4	55.0	64.8	11.7	0.537
				Gall-bladder bile			
494	325	343	30.1	1.4		100.0	0.563
446	287	289	27.6	4.7		29.0	0.576

concentration of total estimated acid radicles exceeded the equivalents of total base, a condition which we encountered in only one instance of all the hepatic biles examined. A very similar condition was found in the gall-bladder bile removed at the time of operation.

Osmotic pressure of hepatic and gall-bladder bile. Determinations of the depression of the freezing point were made with hepatic and gall-bladder bile. Table 5 gives the analysis of the bile specimens used. It is apparent that the osmotic pressure as calculated from the freezing point of hepatic and gall-bladder bile is approximately the same although the concentrations of the constituents of hepatic and gall-bladder bile are at wide variance.

DISCUSSION. The observations reported in this paper were obtained from animals whose gall bladders were receiving known amounts of hepatic bile of determined concentrations. That the gall-bladder wall could function normally under the conditions of the experiment we had previously

determined. The maximum removal of water from the gall bladder we found to be 6.75 cc. per hour. These findings are similar to those of Brugsch and Horsters (1926) who found 6 cc. per hour as the maximum.

The variability of the anion—cation concentrations of hepatic bile in the same animal and in different animals may appear unusual. However, estimations of the pigment concentration of hepatic bile show even greater variations. (Rous and McMaster (1921), Hooper and Whipple (1916), and Riegel, Johnston and Ravdin, to be published.)

Jankau (1891–1892) found the calcium concentration of fistula bile of the dog to vary from 13 to 17 mgm. per 100 cc., and this was not increased by the oral administration of lime salts. Lichtwitz and Bock (1925) found that the calcium content of human fistula bile was from 65 to 84 mgm. per cent of the dry weight and from 85 to 109 mgm. per cent of the dry weight of gall-bladder bile. The calcium content per 100 cc. of fistula bile varied from 4 to 9 mgm. Since these observations were made on bile from exposed fistulae they are open to a number of criticisms. Drury (1924) stated that under ordinary circumstances when the animal was eating well the amount of calcium excreted per cubic centimeter of bile varied little from day to day and from animal to animal. The total calcium output per day varied considerably. Our animals were in good physical condition and ate the regular animal house mixed diet. Although as a rule the variation in the calcium concentration of hepatic bile was not great, low concentrations, considerably below the usual values for serum calcium, were occasionally found. Generally the concentration was above the level of the serum calcium, in some instances reaching twice that level. A study of Drury's data shows that he too found considerable variation in the calcium concentration of hepatic bile in that under normal conditions the concentration varied from 6.1 to 57.8 mgm. per 100 cc. Furthermore, the figures which he gives are as a rule above the concentration of calcium in the serum. We have never observed the high values which he occasionally found in hepatic bile. Whether the calcium is secreted at this high level or whether this might possibly result from the absorption of water by the hepatic ducts we do not know. We believe that the former explanation is more probably correct.

The bicarbonate concentration of hepatic bile was usually considerably higher than that of the serum. In the same animal there were variations of nearly 100 per cent. Drury, Rous and McMaster (1924) found the hepatic bile of the dog to be decidedly alkaline so that Gamble and McIver (1928) inferred that the hepatic bile contained a considerable amount of bicarbonate.

The pH of the hepatic bile of the dog, as determined with the glass electrode described by Stadie, O'Brien and Laug (1931) varied from 7.1 to 8.6±.01. The first studies which we have found in which the hydrogen

ion concentration of the bile is discussed were those of Brand (1902). Since his studies were done so long ago and were done on patients with biliary tract disease no analysis of his data will be given. He does, however, state that fresh liver bile is neutral or alkaline in reaction. Okada (1915) using an electrometric method found an average pH of 7.83 in the liver bile from 16 dogs. Neilson and Meyer, using a colorimetric method confirmed Okada's findings. Their principal studies were done on rabbit's bile.

The bile salts have shown a marked tendency to vary in concentration in different animals, but were fairly constant during similar conditions in the same animal. The chloride concentration varied although the variation was not so marked. The underlying causes for this variation in the anion concentrations is not clearly understood. Such fluctuations do not occur in the serum concentrations from hour to hour or from animal to animal. They may in part be related to the total bile output.

The fluctuation in the anion concentrations was reflected in the total base studies. Gamble and McIver (1928) reported variations in the base of from 157 to 194 m.Eq. per liter in the hepatic bile of the cat. We have found variations of base in the dog's hepatic bile from 174 to 192 m.Eq. per liter. No dry weight determinations were made and therefore no correction for water content of the bile was made.

The discrepancy between total base and determined anions of hepatic bile amounted at the median to 12 m.Eq. per liter. We have called this undetermined anion the "x" anion, realizing fully that it is probably made up of a number of anions. The amount of protein and phosphate present was very low but it is likely that pigment and fatty acids bind some of the excess base. Furthermore the observation that bile that has concentrated for a long period of time in the gall bladder contains more equivalents of acid radicles than of base renders it possible that even where determined anions are less than total base the total acid radicles may exceed the total base and hence the undetermined acid radicles which we have designated "x" anion may be greater than we have calculated.

When the hepatic bile was subjected to gall-bladder activity for a period of from 3 to 8 hours the bile removed was not uniform in the concentration changes which occurred. Base, chloride and bicarbonate generally decreased while pH always decreased. Calcium always, and bile salt generally, tended to increase in concentration. In those instances in which the change in the concentration of the substance was different from that usually attained subsequent experiments in the same animal showed that the results were not due to damage of the gall-bladder wall. We are unable adequately to explain the variations from the usual direction which have occurred. Westphal, Gleichman and Soika (1931) had similar discrepancies from what they considered the usual direction of change

when gall-bladder absorption was influenced by vagus or sympathetic nerve stimulation.

In the seven experiments where base decreased in concentration, anion also decreased. In the two experiments where base increased there was a decrease in the determined anion. The x or undetermined anion concentration increased in seven of the nine experiments. The median of the undetermined anion in these experiments was 26 m.Eq. per liter, an increase of 14 m.Eq. per liter over the median of the undetermined anion in hepatic bile.

Our results indicate that when the empty gall bladder received hepatic bile there occurred first a decrease in the concentrations of cation and total determined anion. During this period the bile salt concentration as well as the undetermined anion concentration increased. A relationship between the sum of the bile salt plus undetermined anion concentrations to the changes in base existed in every experiment. When this ratio was below about 45 per cent total base decreased, but as the ratio approached the 45 per cent level total base again approached its original concentration and finally exceeded it. When these anions accounted for about 45 per cent of the base concentration total base built up. In all instances, however, even though the concentrations were increasing, there was absorption of base and anion when the total amount of base and determined anion in the gall bladder was calculated. This was also true for the individual anions. There was, however, a selective rate of absorption for the different anions, some leaving faster than water while others left at a slower rate.

When hepatic bile was added in small amounts at intervals to the normally functioning gall bladder for a period of from 24 to 48 hours, the samples removed showed a uniform change. Total base and bile salt concentrations increased while chloride and bicarbonate concentrations decreased. The bile removed from the gall bladder frequently contained more equivalents of acid radicles per cubic centimeter than of base. A similar result was obtained when the bile removed from the gall bladder at operation was analyzed. It was obviously impossible to calculate what the concentration of the x anion was in these specimens.

That other anions than those determined were present in small amounts we have shown in several instances. Some protein and phosphate was found, as well as a considerable amount of bilirubin. The results of Hammarsten (1929) indicate that fatty acids are present in hepatic bile and that these are markedly concentrated in gall-bladder bile. This finding probably accounts for a part of the increase of the x anion in the short experiments.

We can only speculate at this time as to the excess in equivalent concentration of acid radicles over base, when hepatic bile is permitted to concentrate in the gall bladder for a long period. The bile acid deter-

minations were expressed in equivalents of cholate measured colorimetrically against a standard of sodium cholate which was taken as monovalent. This calculation of the base binding equivalents of bile acids assumes that whatever the form of bile acids present the color development is proportional to the base binding equivalents. This must be regarded as a tentative approximation which further investigation must test.

The decrease of the pH of hepatic bile when subjected to gall-bladder activity is in accord with the recent observations of Okada (1915), Neilson and Meyer (1921), Drury, Rous and McMaster (1924).

The depression of the freezing point of hepatic and gall-bladder bile is significant Our data indicate that both are approximately the same as that of serum. Coincident with the great increase in concentration of the bile salts there was no increase in the depression of the freezing point. In fact, the depression of the freezing point can be approximately accounted for by the osmolar concentration of the base, chloride and bicarbonate present. Our data are in agreement with those of Brand (1902) and Strauss (1903). Westphal, Gleichman and Soika (1931) give a depression of the freezing point of gall-bladder bile in the atropinized animal of −0.55°C. and state that atropine does not prevent normal absorption. This figure is within the range which we have found.

SUMMARY

The base and the bicarbonate concentrations of hepatic bile are higher than that of serum and the chloride concentration is less than that of serum. Calcium concentration varies but as a rule it is higher than that of serum. The cations and anions all vary in concentration during different periods in the same animal. Chloride and bicarbonate show the greatest variations.

Gall-bladder bile has a higher concentration of base, bile salts and calcium and a lower concentration of bicarbonate and chloride than hepatic bile. The pH of gall-bladder bile is, under normal conditions, always lower than the pH of the hepatic bile brought to it.

Calculated for total amounts both base and the determined anions are absorbed from the gall bladder but the rate of absorption of the different ions varies considerably.

The depression of the freezing point of both gall-bladder and hepatic bile is approximately the same as that of serum so that even though there is a considerable difference in equivalent concentration of solutes present the osmotic pressure remains unaltered.

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SIMULTANEOUS STUDY OF THE CONSTITUENTS OF THE SWEAT, URINE AND BLOOD, ALSO GASTRIC ACIDITY AND OTHER MANIFESTATIONS RESULTING FROM SWEATING

IX. Uric and Lactic Acids

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Adler (1) in 1916, using the Herzfeld colorimetric method, reports quite an appreciable amount of uric acid in the sweat, which is perceptibly increased when the subject is fed on a rich nuclein diet, his figures showing from 3 to 30 mgm. per 100 cc. of sweat. On the contrary Voit (2), using the method of Morris (3), finds there is something under 1 mgm. per 100 cc.

The insufficient amount of data and the wide divergence in the reports of the above named authors have prompted us to make a more exhaustive study of the problem, and as in our previous work we made simultaneous determinations of the uric acid in the blood and urine previous to and after the sweating.

The method of preparing the subject and the means of collecting the sweat, urine, and blood are the same as previously reported by Talbert (4) and his collaborators.

The colorimetric methods of Folin (5), Benedict (6) and Brown (7) were used at different times for making our determinations. All of these methods are essentially the same in that they all depend on phosphotungstic acid for color reaction. However, we found that the Benedict method, as reported by others, gives a slightly higher reading. In the sweat determinations the method of Folin and Brown was used. In the urine determinations we employed the Benedict; while in the blood determinations the Brown method was generally used.

For the determinations in the sweat no dilutions were made other than what the actual analysis entails. We did find it necessary, however, to centrifuge the sweat samples from 5 to 10 minutes at a rate of 2000 r.p.m. to delay precipitation long enough to permit a reading. The values for urine are given in milligrams per 10 cc., for blood in milligrams per 100 cc., and for sweat in milligrams per 1000 cc.

In all ninety observations of uric acid were made of which a few typical

examples are given in table 1. The blood taken after sweating, compared with the control sample, showed an increase in uric acid in 85 per cent of the cases, ranging from 1 to 30 per cent. The urine likewise in 85 per cent of the cases showed an increase in uric acid from 2 to 200 per cent. In thirteen cases, after sweating, samples of urine were taken at hourly intervals until 3 samples were voided. In nine of these cases this increase was maintained for one hour after sweating, and in three of them until the third hour.

This general rise in blood uric acid after profuse sweating may be due to the concentration of the blood, which we have previously shown to occur, rather than to an actual increase of uric acid. But we are disinclined to believe that the slight shift in specific gravity previously noted is a factor of sufficient magnitude to account for the results we have reported. Rather we would stress the rise of body temperature which uniformly obtained in such experiments as the major cause.

TABLE 1

Uric acid in blood and urine before and after sweating

SUBJECT	1ST BLOOD	2ND BLOOD	1ST URINE	2ND URINE	SWEAT
F. B.	2.13	2.13	4.42	3.45	1.19
J. L.	2.12	2.22	3.45	4.13	2.12
F. H.	2.57	3.42	5.68	6.17	1.09
F. C.	3.22	3.81	1.42	3.70	1.39
I. R.	4.03	4.17	2.02	6.94	2.00

It is worthy of note that we found very little uric acid in sweat. Our determinations ranged from 0.72 to 2.52 mgm. per 1000 cc. This is in close agreement with the results reported by Voit. On the other hand they disagree with those of Adler but since our subjects were not on a high nuclein diet they cannot be interpreted to contravene Adler's claim that sweating is a valuable therapeutic measure in certain clinical conditions.

Lactic acid may be found in significant amounts in sweat. In eighty-five observations on twenty subjects we never failed to obtain positive results using the method of Friedemann, Cotonio and Shaffer (8). Typical examples from our data, expressed in milligrams per 100 cc. are: 82.05, 81.36, 71.23, 160.45, 73.46.

SUMMARY

1. Uric acid is constantly excreted in the sweat in small amounts.

2. The uric acid content of both blood and urine rises after sweating. Rise of body temperature rather than blood concentration is preferred to explain this condition.

3. Lactic acid is constantly excreted in the sweat in appreciable quantities.

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A STUDY OF THE EXCISED UTERUS OF THE RAT, ITS VOLUME DISPLACEMENT AND IRRITABILITY TO PITUITRIN, WITH REFERENCE TO THE OESTROUS CYCLE

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In a previous paper (1) we showed a definite relationship to exist between the stages of the oestrous cycle and the spontaneous activity of the excised uterus of virgin rats. Long and Evans (2) measured the horns in different stages and found the diameter to be greatest (5 mm.) in oestrus and least (1.7 mm.) in dioestrus. Osterud (3) weighed the different parts of the reproductive tract of animals of different ages and found that the uterus increased in weight consistently from birth to the 81st day, after which wide variations existed. (These variations occur normally during the sexual life of rats—Long and Evans, 1922.) We (1) have pointed out that the type of response obtained after pituitrin depends upon the pre-existing type of activity, whether normal or artificially induced, i.e., if the rate of contraction is slow, pituitrin (in minute doses) increases it, if maximum rate already exists, the effect is an immediate increase in tonus. These varying degrees of activity are described by Frank, Bonham and Gustavson (4) as resulting from the influence of the female sex hormone. Since this hormone is inhibitory in action (4) (5) we must deduce that its absence would result in a hyper-activity, and vice versa. It is apparent that something, exerting influence upon the irritability of the uterus to pituitrin, accompanies the cyclic changes. This will be discussed more fully presently. Durant and Rosenfeld (5) confirm Frank and his collaborators and report the effect as being primarily upon the rate and not the amplitude of contraction. Childs (6) suggests that the difference in activity found in isolated portions of the horns may be due to a gradient of metabolism. Experimental evidence following up this suggestion has not yet appeared; however, some recent work on the metabolism of whole excised horns was reported by Khayyal and Scott (7) who found active metabolic processes in the excised uterus of both the rat and the mouse, the oxygen consumption being greatest before oestrus, and constant at a lower rate during dioestrus. The mechanism controlling these metabolic changes points to the hormones associated with oestrus. When oestrus is artificially induced the oxygen consumption increases as oestrus ap-

proaches (7). The relation of oxygen consumption to spontaneous activity appears quite definite in dioestrus, but not so convincing in the first stages of the cycle. This deviation may be accounted for in the fact that in stages I and II uterine secretion raises the oxygen requirements. On this phase of uterine function more work is needed.

In this paper it is desired to carry these investigations farther and study the uterine activity as it relates to volume displacement, relative and absolute length of horns, surface area and the influence of end-portions of the horns, upon spontaneous activity. Pituitrin in minute doses (0.01 cc. in 50 cc. of Locke's solution) is used to test the irritability. A system of ligation (fig. 1) is used making possible manipulation of the tissue at any time during the experiment.

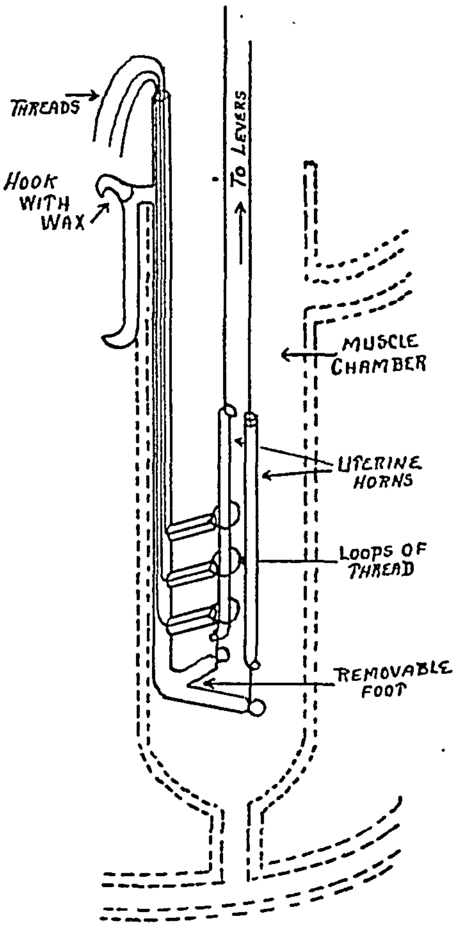


Fig. 1. Showing the system of ligation used. One horn is made to contract through a series of loops, each communicating with the outside, and may be tightened at will. The other is free.

Apparatus. The apparatus previously described (1) was used throughout with the addition of an especially adapted foot facilitating manipulation (see fig. 1).

Animal material. All animals were virgins in good condition and were selected for age and weight.

Procedure. The following additions to our original (1) were made. 1. Whole horns were first tied off at both ends, then cut free of their attachments and removed to the apparatus of Rubinstein (8) containing Locke's solution for volume displacement determinations, after which they were immediately transferred to the muscle chamber. 2. During the interval of relaxation (i.e., between contractions) the horns were measured under the stress of the writing lever (1 gram). 3. The tissues were given 30 minutes to establish their typical responses. 4. Finally, after the tissues had been experimented upon they were returned to fresh oxygenated Locke's solution and subjected to 5 times the stress of the lever (5 grams) and again measured for absolute length.

EXPERIMENTAL. For convenience these experiments may be divided into 3 groups: 1. The influence of ligating end-portions of the horns on spontaneous activity. 2. The relation of volume displacement and surface area to spontaneous activity. 3. The relation of volume displacement to pituitrin action.

1. *The influence of ligating end-portions of the horns upon spontaneous activity.* It is well known that if the uterus is bisected the two portions will contract independently but at different rates (1), (5). The ovarian end more closely represents the type of activity predicted for the whole horn (1). All these responses, however, are influenced by the oestrous cycle (1). One question arising out of recent observations we have attempted to answer here, viz: Where does the influence of the tubal end stop? In figure 2 the activity of 8 horns is reproduced, 4 exhibiting activity typical of the stage in which they were excised, and 4 sister horns subjected to various types of ligation. In curve I, 1 cm. of the vaginal end was ligated. Following one powerful response, irregular but otherwise typical contractions occurred accompanied by the characteristic fall of the tonus line. One may consider it a curve of reduced activity due to removal of a portion of the contractile tissue. In curve II a 3 mm. portion of the tubal end of the right horn was ligated before removal, while the left horn was kept intact. Over a period of 30 minutes no maximal contractions occurred in the ligated horn, while the normal (left) executed the characteristic responses. In curves III and IV the horns were removed and suspended in the usual fashion, and allowed to start their typical response, after which they were ligated to different extents, and at different time intervals. It was found that following this treatment inhibition resulted, the duration of which showed a definite relation to the oestrual stages. In the pre-oestrual stages (lt. V, I and ea. II) the inhibition may last a long time (30 minutes), while in the more active stages (III, IV, and ea. V) it is quite transitory, seldom lasting longer than 5 minutes.

The recovery from ligation and the type of return contraction are influenced by the amount of tubal end involved. In stages I and II characteristic inhibition can be induced by ligating very small portions (3 mm.), while in the more advanced stages 1 cm. may be required (curves II, III and IV, fig. 2).

The return contractions are difficult to analyze. They are never like the original in either rate or amplitude, but irregular, and occasionally compound in nature. They present possibilities, however, in that their normal controlling mechanism is apparently absent, and in this way more completely susceptible to environmental influences.

The tubal end influence may be completely removed in any stage of the oestrous cycle by ligating 1 cm. of the horn. The remainder of the horn will respond in a manner typical of the vaginal end of the uterus.

These experiments point to the possibility that the irregular curves often observed may be the result of ligation or destruction of the tubal

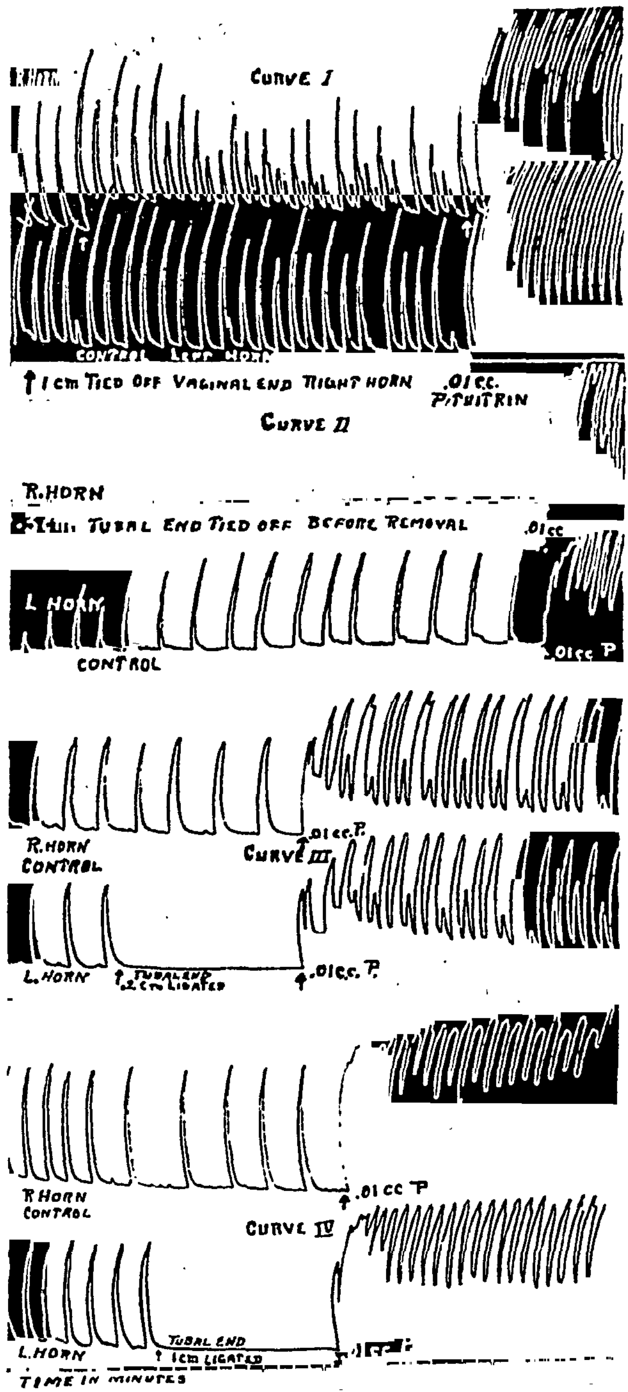
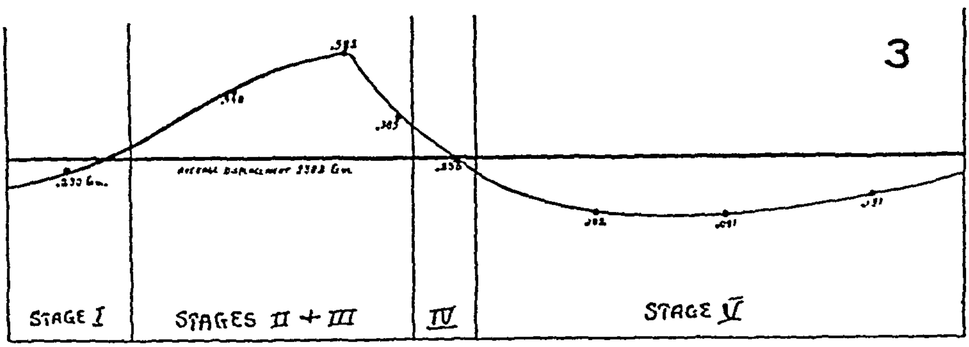


Fig. 2. Showing normal responses, and effect of various types of ligation upon 4 pairs of horns from virgin rats of equal size, and condition. The records of each pair were made simultaneously.

end of the horn, and suggest that the tubal end may exert upon uterine activity a controlling influence, which if cut off results in irregularity of response, and a reduced rate of contraction.

2. *The relation of volume displacement and surface area to spontaneous activity.* The curve in figure 3 represents the volume displacement of whole uterine horns of animals of approximately the same weight (178 grams, plus or minus 5 grams), excised at various stages of the oestrous cycle. The uterus was found to be large during oestrus and small in dioestrus, confirming Long and Evans (2). The change from one level to the other is relatively abrupt, but more pronounced during regression.



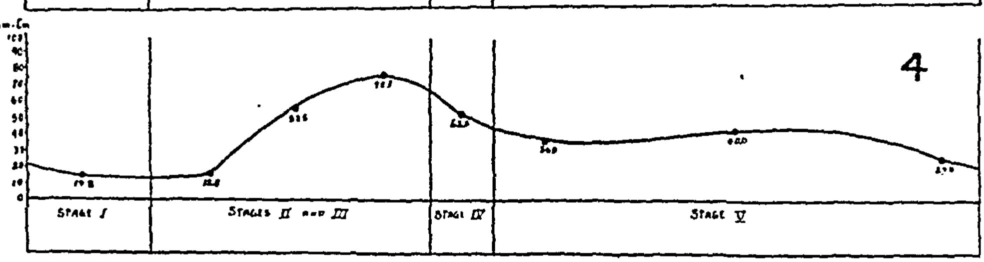


Fig. 3. Showing volume displacement of left uterine horns of virgin rats with reference to the oestrous cycle. Weight 178 grams (plus or minus 5 grams). Vertical lines divide the stages.

Fig. 4. Showing the curve of uterine activity, expressed in gram-centimeters of work, for left horns of virgin rats weighing 165 grams (plus or minus 3 grams) with reference to the oestrous cycle.

The average displacement (0.260 gram of Locke's solution at 25°C.) is present only momentarily, occurring in stage I and again in stage IV or early V. The displacement of the uterus is above average for about $\frac{1}{3}$ (30 hours) of the oestrous cycle (stages I, II, III and IV) and below average about $\frac{2}{3}$ (70 hours) of the cycle (stages IV, V and early I). These changes in volume displacement became interesting when compared with the curve of uterine activity.

In figure 4 the curve of spontaneous activity is plotted from left horns of 8 animals (nos. 2, 3, 5, 6, 10, 12, 14, and 16, table 1) upon the same scale of hours as figure 3. The activity is expressed in gram-centimeters of

work per hour. The curves practically coincide in the ascending phase, but diverge as they descend. We must deduce from this that while there is regression taking place there is also a force maintaining spontaneous activity, but at a submaximal level. The curves, which in stage II rose together, take opposite directions for a period in dioestrus. This means that volume displacement or tissue mass cannot account for the varying degrees of activity observed in the different stages of the oestrous cycle, and points to other phenomena.

TABLE 1

Showing the relation between length of horns, volume displacement, and work, with reference to the oestrous cycle

ANIMAL NUMBER	WEIGHT	STAGE	LENGTH OF HORNS 1 GRAM STRESS		ABSOLUTE LENGTH OF HORNS 5 GRAMS STRESS		VOLUME DISPLACEMENT		WORK	
			Left	Right	Left	Right	Left	Right	Left	Right
	grams		*cm.*	*cm.*	*cm.*	*cm.*	*grams*	*grams*	*grams-cm.*	*grams-cm.*
1‡	173	I	4.2	4.3	4.3	4.4	0.230	0.259	21	21
2§	164	I	4.0	4.2	4.1	4.3	0.222	0.230	14.8	17
3§	168	II Ea.*	4.2	4.2	4.3	4.5	0.450	0.474	12	12
4‡	178	II	4.2	4.4	4.3	4.5	0.440	0.445	31	34
5§	168	II	4.0	4.5	4.2	4.6	0.563	0.574	55.5	63
6§	164	III Ea.	3.9	4.2	4.1	4.4	0.574	0.570	75.3	81
7‡	183	III	4.2	4.3	4.4	4.4	0.582	0.580	70	72
8‡	180	III Lt.†	4.2	4.1	4.4	4.3	0.385	0.378	50	46
9‡	183	IV	4.5	4.6	4.7	4.7	0.255	0.255	54	57
10§	162	IV	3.9	4.1	4.3	4.5	0.269	0.267	62.5	60
11‡	184	V Ea.	4.1	4.3	4.3	4.5	0.102	0.101	39	40
12§	162	V Ea.	3.7	4.0	3.9	4.2	0.100	0.108	36	40
13‡	182	V	4.1	4.3	4.3	4.6	0.091	0.102	38	40
14§	164	V	3.9	4.0	4.3	4.5	0.081	0.090	40	40
15‡	178	V Lt.	4.1	4.3	4.3	4.6	0.131	0.120	32	32
16§	164	V Lt.	3.8	3.8	4.1	4.2	0.140	0.154	24	25

* Ea. = Early.

† Lt. = Late.

‡ Animals used in plotting the curve of volume displacement.

§ Animals used in plotting the curve of uterine activity.

3. *The relation of volume displacement to pituitrin action.* From the beginning we noticed that the uterus of certain animals of our series was sensitive to minute doses (0.01 cc. in 50 cc. of Locke's solution) of pituitrin, while others tolerated that amount with little evidence of the typical effect. Three possibilities present themselves. 1. The surface area exposed to pituitrin was not always the same. 2. The irritability of the uterus may follow the curve of spontaneous activity. 3. The activity may be controlled by a hormone (4).

Toward the solution of these problems additional experiments were performed and our older experiments again analyzed. Horns of the same absolute length (measured under 5 grams stress after the experiment), but in different stages of the oestrous cycle were selected from the lot for analysis here (animals 1, 4, 11 and 15, table 1). The results are plotted in figure 5. It was found that while the maximal height of contraction is maintained throughout stages I, II, III, and IV (C, fig. 5), in stage V it is decreased per unit length of horn. This result is so consistent in stage V that we must attribute it either to some inhibiting process, or to the absence of an activating substance. Our experiments point to the former.

When these horns are subjected to small doses (0.01 cc.) of pituitrin the response is entirely independent of tissue mass or surface exposure.

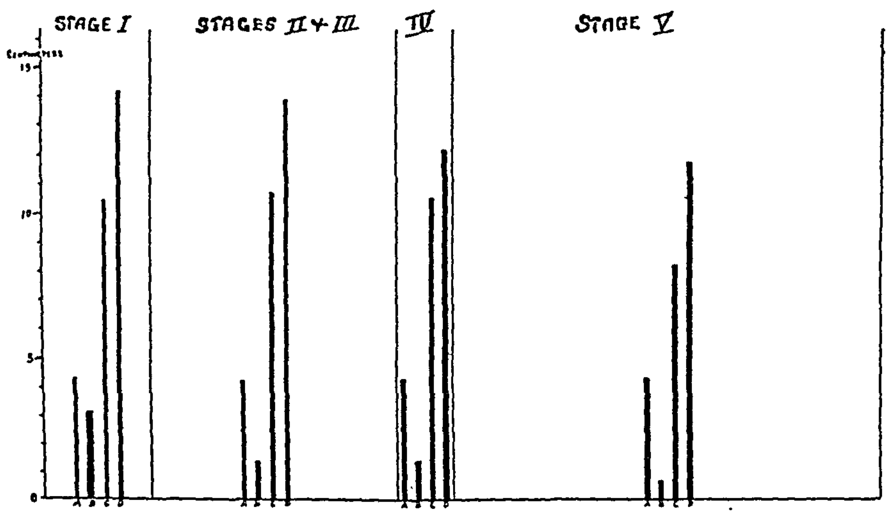


Fig. 5. Showing the relation of spontaneous activity of excised uterine horns of virgin rats, before and after small doses of pituitrin "O" (0.01 cc.) to the stages of the oestrous cycle. A, length of horn; B, tonus rise after pituitrin; C, normal height of maximal contractions; D, height of maximal contraction after pituitrin (0.01 cc.).

In stage I, where the volume displacement is average or below, the response is greatest (B and D, stage I, fig. 5). In stages II and III the displacement is far above average, but the effect upon tonus is much reduced (B, stages II and III, fig. 5). Stage IV presents an additional variation—the rise in tonus remains, but the tissue does not contract to a maximum (D, stage IV, fig. 5). A combination of these effects is found in stage V. The tonus increase is slight. The ability of the tissue to shorten is increased, but is still incomplete. As this stage approaches stage I, the tonus is much increased, and the contractions become maximal. In midstage V it seems that the pituitrin action is chiefly expressed in the building up of stunted maximal contractions. From the gross aspect it seems possible that the difference may be due to the small surface area exposed to the

drug, but this is dispelled upon closer analysis. If the dose of pituitrin be increased by 0.001 cc. (10 per cent, all the characteristic responses return, the tonus rise is increased, and the horns completely shortened. Since the volume displacement in stage V is often only $\frac{1}{6}$ and the surface area 32 to 40 per cent of that in stage II, the slight increase in dosage would be ineffectual if either surface area or volume displacement was a chief factor. It seems more probable that there exists some substance the function of which results in the volume changes observed, and bears a definite relation to pituitrin action and oestrual changes. The presence of this substance produces uterine inhibition with a low threshold to pituitrin, and vice versa. It is only by this premise that we can explain our results, and correlate the evidence at present in hand. This substance is in its highest concentration in stage I and gradually diminishes through the succeeding stages to late stage V where it shows recovery, and the cycle repeats. Upon the activity of this substance the curves of uterine activity, volume displacement, oxygen consumption, and the variable response to pituitrin can be explained and correlated with respect to the cyclic function of the reproductive tract.

CONCLUSIONS

1. Volume displacement of uterine horns has no certain relation to spontaneous activity.
2. The volume displacement of horns of virgin rats weighing 173 grams (plus or minus 11 grams) ranges between 0.081 gram in stage V to 0.582 gram in stages II and III.
3. The variation in the response of excised uterine horns to small doses of pituitrin cannot be explained on the basis of surface area exposed.
4. There exists in excised uterine horns of the virgin rat a graded irritability to pituitrin. The irritability decreases as the stages advance, i.e., it is highest in stage I and lowest in stage V.
5. Irregularity of response in excised horns is doubtless due to injury or absence of the tubal end of the horn. In stage III the influence of ligation is least, while in stage I it produces long intervals of inhibition.

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STUDIES ON THE PHYSIOLOGIC ACTION OF RATTLESNAKE VENOM (CROTALIN)

XI. Effect of Crotalin on Swine

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In many localities the belief is current that swine possess a natural immunity to the venom of poisonous snakes. It is claimed by many farmers that rattlesnakes will not live in fields frequented by hogs since the latter attack snakes, kill and eat them, and that hogs are not harmed since they are immune to the venom of the snake. The desire to test the validity of such statements prompted this study.

Previous studies have shown that the injection of crotalin into dogs and rabbits produces a marked fall in blood pressure and causes a decided increase in the volume of the erythrocytes in vivo or in vitro, accompanied by more or less hemolysis. The susceptibility of a given animal to crotalin may be judged by the response of the animal's blood pressure and erythrocytes to a standard dose of this venom. The effect of crotalin on swine is illustrated by the following experiments.

A shoat, aged five weeks, weighing 6.7 kgm., was etherized and the carotid blood pressure recorded in the usual manner. An intravenous injection of 0.03 cc. of 2 per cent crotalin (Crotalus horridus) for each kilogram of body weight, which is about half the usual lethal dose for the dog, was made into a vein of the ear. Immediately following the injection the blood pressure fell rapidly from 100 mm. to 30 mm. of mercury. Twenty minutes later it began to rise and had reached a physiologic level in about thirty minutes. The same dose was repeated and a similar response resulted but recovery was delayed. Twenty-four minutes after the second injection the blood pressure had risen to only 50 mm. of mercury where it remained until the tracing was discontinued fifty minutes later (fig. 1). The shoat gradually recovered and was walking about and taking food sixteen hours after the experiment

To determine the effect of the venom on the erythrocytes, a sample of blood (11 cc.) was drawn into heparin immediately before the first injection and six minutes after the second injection. These specimens were centri-

fuged for twenty minutes at about 1,500 revolutions each minute. The volume of the erythrocytes in the first tube was 5 cc. and there was no hemolysis, whereas the volume of the erythrocytes in the second tube was 8.2 cc. and considerable hemolysis was present. As is true with the dog the clotting time of the blood of swine is prolonged following an injection of crotalin. In one instance the blood was still fluid after sixteen hours.

Twelve days after the experiment described, the shoat was given an intravenous injection of 1 mgm. of dried venom for each kilogram of body weight. This was followed almost immediately by complete prostration. The breathing was very shallow, the pulse was rapid and thin. The symptoms differed from those of the dog since there was absence of vomiting, urination and voiding of bloody feces. Within an hour the shoat was walking about apparently recovered. The next morning, however, it was found dead. At necrospy the jejunum was full of blood. The lungs were

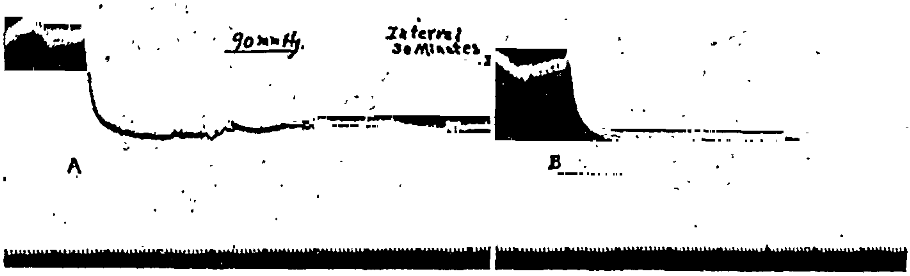

Fig. 1 *a*. The results of two successive intravenous injections, A and B, of 0.03 cc. of 2 per cent rattlesnake venom for each kilogram of body weight in a pig weighing 6.7 kgm. Time in intervals of five seconds.

distended and the heart showed a few petechial hemorrhages in the auricles, and in the ventricles were areas of subendocardial hemorrhage near the bases. As occurs in the dog, the bladder was extremely contracted. The blood in the heart and large vessels was fluid. Similar results were obtained in other experiments. In one instance a shoat succumbed to a dose of 0.03 cc. of 2 per cent crotalin for each kilogram of body weight but in all other experiments a much larger dose was required to produce death. An intravenous injection of 1 to 1.5 mgm. of the dried venom for each kilogram of body weight might be expected to prove fatal in a high percentage of cases.

COMMENT

The physiologic responses of the pig are similar to those of the dog following an intravenous injection of a lethal dose of crotalin. The characteristic fall in blood pressure, accompanied by a decided increase in the

volume of the erythrocytes, and the post-mortem observation, resembled closely those noted in other susceptible animals.

It is probable that the thick layer of subcutaneous fat usually possessed by swine may sometimes retard rapid absorption of the venom following a bite, and thus acute poisoning may be prevented. However, it is safe to conclude in the event a hog is bitten by a poisonous snake, serious symptoms may reasonably be expected to follow if a sufficient quantity of venom has been injected. It is apparent that swine are not more immune than other domestic animals to rattlesnake venom.

THE FAILURE OF DIET TO INFLUENCE THE ADRENIN CONTENT OF THE ADRENAL GLANDS

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Cannon and Uridil (1921) found in cats a greater output of a cardio-accelerator substance, when they stimulated the nerves of the liver, if the animals were digesting protein. Since adrenin, sympathin, and the substance liberated from the liver have similar physiological effects (Rosenblueth and Cannon, 1932), it was deemed interesting to learn whether diet influences the adrenin content of the adrenals.

Rats were chosen because they are omnivorous. Lots varying from 5 to 15 were put on different diets for varying periods (8 to 35 days). Four lots received only lean meat and water as food; three others only water and beef-fat; three others only water and sugar; one lot, finally, had only water for eight days.

The animals were killed by a blow on the head. They were bled by cutting the carotids. The glands were immediately extirpated, weighed and ground with sand and hydrochloric acid. The adrenin content was determined by means of the colorimetric method of Folin, Cannon and Denis (1913).

The following results were obtained:

DIET	NUMBER OF DAYS	NUMBER OF RATS	WEIGHT OF RATS BEFORE DIET	WEIGHT OF RATS WHEN KILLED	WEIGHT OF ADRENALS	ADRENIN PER 1 GM. OF GLAND
			grams	*grams*	*mgm.*	*mgm.*
Protein	8	5	670	680	230	3.5
	8	11	1,980	2,028	480	3.3
	15	11	2,430	2,480	420	3.4
	35	10	1,030	1,318	350	3.9
Fat	8	5	670	660	110	2.6
	8	12	2,580	2,596	710	3.1
	35	13	1,482	1,600	500	3.6
Sugar	8	6	620	612	140	3.2
	8	11	1,710	1,668	470	4.1
	35	15	2,083	1,846	500	4.5
Fasting	8	7	1,087	1,027	310	1.8

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[2] Fellow of the Rockefeller Foundation.

These results show that diet has no consistent influence on the amount of adrenin in the adrenals of the rat.

The control observations on fasting animals confirm the results reported by Venulet and Dmitrowsky (1910), Ogawa (1925) and others, i.e., a lower content of adrenin.

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THE CURRENT FLOWING THROUGH THE HEART UNDER CONDITIONS OF ELECTRIC SHOCK

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In studying the effect of electric shock upon rats (1, 2, 3) it became evident that the injury was in the main confined to the portion of the body that was traversed by the current. The present paper is directed specifically to a study of the effect upon the heart since this organ is believed to be especially liable to injury in electric shock. The purpose of the present experiments was two-fold; to ascertain the proportionate value of the total current which actually flows through the heart when contact at various points on the body is made with the circuit and to determine the minimum current necessary to establish ventricular fibrillation.

Dogs, completely anesthetized with morphia and ether, were used as experimental animals because the ventricles of the dog's heart are readily thrown into a permanent state of fibrillation by the application of relatively weak currents as is assumed to be the case in man.

The source of the current was a 60 cycle, alternating current circuit. Ring or through type current transformers were employed to measure the current traversing the heart, the heart itself forming the primary winding of the transformer. The iron cores of these transformers were ring shaped and of the highest quality magnetic material.[1] They were approximately square, having a cross-sectional area of 0.01 square inch (0.07 sq. cm.). Each ring was uniformly wound with approximately 2000 turns of no. 36 Brown and Sharp enamel insulated copper wire which served as the secondary winding on the transformer. The rings were of varying inside diameters, ranging from $\frac{5}{8}$ inch up to $3\frac{1}{2}$ inches, so that they could be slipped over hearts of different sizes. The current flowing through the heart induces an electro-motive force in the secondary winding of the transformer encircling it. The terminals of this secondary winding were connected across a potentiometer type rheostat, and the desired portion of the voltage drop in this rheostat was fed into an amplifier whose

[1] The authors wish to take this opportunity to thank the Bell Laboratories Inc. and the Westinghouse Electric and Manufacturing Company for their kindness in furnishing the transformer cores.

output was a measure of the current flowing through the heart. The complete details of the electrical equipment are given in another paper by one of the authors (4).

In order to check the operation of the ring transformers they were immersed at the center of a conducting bath consisting of a ten per cent sodium chloride solution. The bath was over six feet in length and approximately five by five inches in cross-sectional area. In a bath of these dimensions, placing the electrodes at the extreme ends insures a uniform current distribution at the center. Tests proved that when the plane of the ring was perpendicular to the direction of the current flow, the amplifier reading measured the total current flowing through the area inclosed by the ring. When the ring was turned so that its plane was parallel to the current path the amplifier reading was zero. In all of the tests it was found that the amplifier output was equal to the current actually flowing through the area inclosed by the ring. Therefore, with the ring inclosing the heart it measured the current through that organ.

Application of transformer. The thorax was opened under artificial respiration and the pericardium freed from its attachment to the diaphragm. A ring transformer of suitable size was passed over the apex until it fitted snugly about the mid-region of the ventricles where it was held in place by ligatures passing through the pericardium. After the ring was in place the pericardium was sewed closely to its original attachment on the diaphragm. The fully insulated lead wires were carried to their proper connections and the thorax was then roughly closed, to establish *approximately* normal conditions.

The heart is such a mobile structure that it is difficult to determine its electrical conductivity at any single instant. Its only permanent connection is with the great vessels at its base. During expansion of the lungs they make good contact at the sides, a contact which is less perfect during expiration. The apex of the heart lies in proximity to the diaphragm but the effectiveness of contact at this point varies continually. Moreover the operation upon the chest caused a further change in these normally variable conditions. By closure of the chest after insertion of the ring and applying mechanical inflation and deflation of the lungs throughout the experiments, physiological conditions were preserved as carefully as possible.

The equipment controlling the voltage supplied to the electrodes was such that any desired value of voltage could be obtained and the total current passing through the body held constant. An ammeter inserted in the circuit measured the total current.

Period during which current distribution is constant. With different points of contact on the body the amount of current passing through the heart could be determined upon the animal after death provided no appre-

ciable change developed in the current distribution through the body. Figure 1 shows the results of one experiment. In this case the total current through the animal (head to tail) was held constant by controlling the voltage of the supply circuit. Artificial respiration was continued even after the heart ceased to beat and the current flowing through the heart varied slightly, presumably with the amount of air in the lungs, but it is apparent that satisfactory constant conditions prevail for at least thirty minutes.

With these data in hand, which were confirmed in other experiments, it was felt justifiable to continue observations on the dead animal for the period specified in order to conserve material.

In a number of experiments the total current flowing through the body was varied from a low value to several hundred milliamperes, and the current flowing through the heart noted. The heart current proved to be a fixed percentage of the total current flowing.

The change in the current distribution apparently coincides fairly closely with the onset of complete rigor mortis. It was noted in the experiments

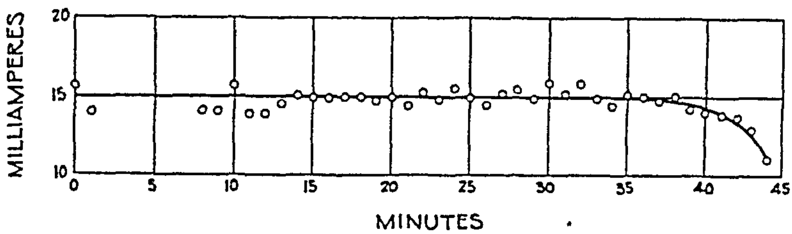

Fig. 1. Variation of current through heart with time following the death of the animal.

that rigor of the neck musculature was judged to be complete one-half hour after death.

Current passing through heart for different current paths. Tests were made on a number of animals with the ring transformers encircling the heart, thus measuring the current that flowed through the heart for various current paths. In these experiments the total current flowing through the body was varied in the different animals, but in any given animal it was held constant during the test. The electrodes were first placed on the head and tail. The total current through the animal was adjusted to the desired value and that through the heart read on the amplifier. The electrodes were then shifted to a new position, the total current through the dog readjusted to the given value and that through the heart measured. The electrodes were then shifted to the next position and the procedure repeated. When all the important current paths had been tried, the electrodes were shifted back to head and tail and a second series begun. It was possible to obtain four to five series of readings on each

animal during the half hour period before rigor mortis set in. Thus each value of heart current for a given current path found in table 1 represents the average of four or five separate readings taken at intervals of six or seven minutes. In some of the tests only a few current paths were studied and in these cases the data in the table are the average of a larger number of readings.

It is evident from table 1 that there is a fair agreement between the results obtained with the different animals when the current flowed downward through the trunk. However, for points of contact where the current path was at right angles or nearly so to the trunk the agreement is

TABLE 1

Ring encircling heart

	Weight	Total current	Percentage of total body current passing through heart for current pathway from:												
			Head to tail	Head to right hind-leg	Head to left hind-leg	Right fore-leg to left hind-leg	Right fore-leg to right hind-leg	Left fore-leg to left hind-leg	Left fore-leg to right hind-leg	Right fore-leg to tail	Left fore-leg to tail	Right hind-leg to left hind-leg	Head to right fore-leg	Head to left fore-leg	Right fore-leg to left fore-leg
	pounds	*milli-amperes*													
1	8	160	9.0												
2	8	55	9.0												
3	9.5	160/235													0.8
4	9	150	7.7	8.4	8.8	—	—	6.6	6.4	—	6.9	0	5.5	3.9	—
5	10.7	150	8.5	10.1	10.0	6.8	7.1	8.8	8.5	7.0	8.8	0	3.1	1.0	1.3
6	9.3	100	8.8	8.7	8.3	7.5	7.7	6.3	6.5	7.5	6.3	0	6.6	0	6.7
7	7.5	150	7.5	—	—	6.2	6.2	—	—	7.0	—	0	3.9	1.1	4.7
8	11.0	150	9.7	10.8	9.8	8.3	8.3	8.0	8.6	7.7	7.6	0	0.5	0.5	0
9	11.3	120	10.5	10.4	10.3	9.5	10.3	5.5	5.3	9.1	4.7	0	0	4.3	3.9
Average.........			8.8	9.7	9.5	7.7	7.9	7.0	7.1	7.7	6.9	—	3.2	1.8	2.9

poor and there is a wide variation in the values obtained with different animals. The lack of agreement is probably due to several causes, the principal one being the difficulty of restoring complete normal conditions when closing up the opening in the chest cavity.

A study of the results in table 1 shows that approximately nine per cent of the total current flowing down through the trunk from the head to the lower extremities passed through the heart. It is interesting to note that more current flowed through the heart when the current entered the right fore-leg and passed out at either hind-leg or tail, than when it entered via the left fore-leg. These results check the earlier experiments (2) made on rats when it was found that only 33 per cent recovered when the current

path was from right fore-leg to tail, while 58 per cent recovered when the left fore-leg was used in place of the right. It was found that when the current path was from hind-leg to hind-leg, no current flow through the heart could be detected. This result also confirms experimental data found with rats (2).

The last three columns of table 1 give the data for the cases where the current pathway was from the head to either fore-leg or from one fore-leg to the opposite fore-leg. When contact at the body is made at these points the current flows more or less transversely through the thorax. As already pointed out, the agreement here is poor. A few experiments were made with the two inch ring transformer sewed along the right side of the heart, thereby fixing its plane parallel to the body axis. The current path in these tests was between the fore-legs and the average current flowing through the ring was found to be three per cent of the total current in the circuit. This value also checks closely that found with the transformers encircling the heart.

Current necessary to initiate fibrillation. In seven experiments the current through the heart necessary to initiate fibrillation was measured. The electrodes were attached to the head and tail of the animal, and the total current was gradually increased using a five second shock at each value. The current actually flowing through the heart for each shock was determined from the amplifier output. After each five second shock the thorax was opened and the condition of the heart visually determined. There was considerable variation among the individual animals as was to be expected. In one case a heart current of six milliamperes caused fibrillations, in another the value was 15 milliamperes. The average value was 8.6 milliamperes.

Fibrillation experiments. In order to check the accuracy of the results several tests were made to find the amount of current that must be applied to the surface of the body with different leads in order to produce ventricular fibrillation without the ring transformers in place. In these tests one electrode was kept permanently connected to the right fore-leg while the other was connected to either the left fore-leg or to the two hind-legs which were used as a single terminal.

Two experiments were made with the heart perfused *in situ.* With the current pathway down through the trunk, the current through the animal was increased until fibrillation was initiated. Then the heart was recovered by the injection of potassium chloride into the coronary arteries (5). Next the movable electrode was transferred to the left fore-leg and the current now flowing transversely through the body adjusted to the value that would initiate fibrillation for this current pathway.

The results of one of these experiments are given in table 2. These data show clearly that for this animal a total current of 90 milliamperes

flowing in at the right fore-leg and out at the lower extremities was enough to set the heart in fibrillation. With the current flowing transversely between the fore-legs it required 243 milliamperes to start fibrillation.

The data in table 1 gave the average heart current as 7.8 per cent of the total body current for a current pathway from the right fore-leg to either

TABLE 2

DURATION OF SHOCK	TOTAL BODY CURRENT	POSITION OF ELECTRODES	HEART RESPONSE
Blood flowing normally through heart, thorax open, artificial respiration			
seconds	*milliamperes*		
5	92	Right fore-leg. Left fore-leg	No fibrillation
5	88	Right fore-leg. Hind leg	Fibrillation. Heart recovered
Heart perfused with oxygenated Locke's solution. Artificial respiration continued			
5	85	Right fore-leg. Hind leg	Fibrillation. Heart recovered
5	91	Right fore-leg. Left fore-leg	No fibrillation
5	103	Right fore-leg. Left fore-leg	No fibrillation
5	125	Right fore-leg. Left fore-leg	No fibrillation
5	83	Right fore-leg. Hind leg	No fibrillation
5	100	Right fore-leg. Hind leg	Fibrillation. Heart recovered
5	110	Right fore-leg. Left fore-leg	No fibrillation
5	135	Right fore-leg. Left fore-leg	No fibrillation
5	176	Right fore-leg. Left fore-leg	No fibrillation
5	220	Right fore-leg. Left fore-leg	No fibrillation
5	243	Right fore-leg. Left fore-leg	Short fibrillation, then normal. Heart recovered
5	102	Right fore-leg. Hind leg	Fibrillation. Heart recovered
5	79	Right fore-leg. Hind leg	No fibrillation
5	83	Right fore-leg. Hind leg	Fibrillation. Heart recovered
Heart beat feeble			
5	155	Right fore-leg. Left fore-leg	No fibrillation
5	79	Right fore-leg. Hind leg	Fibrillation

hind-leg. In this experiment using the same current path it required 90 milliamperes flowing through the animal to initiate fibrillation. This was equivalent to a current through the heart of seven milliamperes. In the cases where the current path was from fore-leg to fore-leg the current

through the heart was approximately 3 per cent of the total current, which corresponded in this check experiment to 7.3 milliamperes through the heart. This test was successfully repeated several times and the current values check closely, as may be seen from table 2. The results obtained with the other animal tested in this manner were equally good.

SUMMARY

It is evident from these experiments that 9 or 10 per cent of the total current passing through the body flows through the heart for a current pathway parallel to the body axis. When the current flows transversely only about 3 per cent passes through the heart. Thus as far as the heart is concerned fibrillation will be produced by a much smaller total current flowing from the upper to the lower extremities than between the fore-legs.

In most industrial accidents the current path is from right hand to the feet and under these conditions the heart carries a greater proportion of the total current than when contact with the circuit is made at any other location on the body.

The authors wish to take this opportunity to acknowledge their appreciation to the Committee on Physiology of the Conference on Electric Shock for providing funds for this work.

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THE DIGESTIVE LEUCOCYTOSIS QUESTION

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In 1854 Moleschott (1) described digestive leucocytosis which is said to be particularly noticeable after a meal of protein. Von Limbeck (2) asserts its existence, as do Arneth (3) and many others. The phenomenon has been uncritically accepted as a part of the physiologist's *credo*, but there have always been dissenters and in recent literature one frequently finds the interjection of a note of doubt (4, 5, 6). It may be questioned whether any really adequate investigation of the phenomenon has as yet been made owing to a lack of appreciation of the number of factors and the sensitiveness of the mechanisms which cause variations in the number of circulating leucocytes. The control of these variables is essential to a correct interpretation of experimental results and this is the objective in the experiments reported in this communication.

It is first of all necessary to establish an unvarying norm for the leucocyte count under conditions which permit the induction of one variable at a time, just as one compares all metabolic variations to the basal metabolic rate. Preliminary experiments (7) have demonstrated that the leucocyte count, made on blood obtained by deep, sharp puncture of either the finger or ear, of over two hundred students and staff members, pursuing ordinary laboratory activities, usually lies between 8000 and 9000 per cubic millimeter and this has been designated the *activity level*. The count may be somewhat higher or slightly lower depending on the individual and the degree of activity. Upon assuming the recumbent posture and maintaining a condition of absolute rest the count falls rapidly. In approximately an hour it reaches its lowest level which remains constant for hours as long as the state of rest and immobility are maintained. We designate this the *basal* leucocyte level. Numerically it usually lies between 5000 and 6000 leucocytes per cubic millimeter, although it varies somewhat with different individuals and occasionally may be somewhat above or below these figures (7, 8). A rise above the basal level constitutes a physiological leucocytosis and may be the accompaniment of extremely slight alterations of physiological state.

The first experiments were conducted on subjects in the basal metabolic state, in the early morning and without food. The results of six such

experiments are illustrated in figure 1, A and B; they are typical of all. Leucocyte counts were made at the activity level and every fifteen minutes after assuming the recumbent resting condition. After an hour when the basal level was reached a heavy protein meal was fed to the passive, immobile subjects. The meal consisted of one and a half to two pounds of beef steak, two to four eggs and one to two slices of toast, depending upon the capacity and willingness of the subject; no fluid was administered. The fifteen minute counts were continued for two to four hours—as long as the subjects could be kept quiet. There was not a single instance in which the leucocyte count rose above the basal level while the subject remained at rest. The authors consider that this constitutes a crucial experiment indicating that the processes of digestion and absorption *per se* do not cause a leucocytosis. Whenever, in the experiments under consideration, the subject assumed an erect posture there ensued an immediate

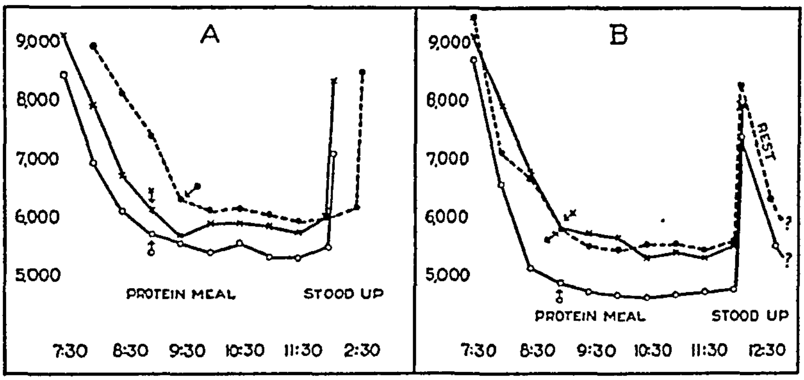

Fig. 1. Failure to obtain a digestive leucocytosis. Two groups of curves, A and B, three individuals in each group, showing 1, that recumbent rest decreases the leucocyte count to a basal level; 2, that a heavy protein meal, administered at the points marked by arrows, did not cause a rise in the basal level. The counts were made at fifteen minute intervals.

rise in the leucocyte count after which recumbent rest was followed by the gradual fall of count to the basal level again in spite of the progress of the digestive process.

In a similar way carbohydrate meals were fed, consisting of approximately five pancakes and about 150 cc. or 200 cc. of a well known brand of syrup. The test conducted as described for the protein meal gave an identical result and the graphs in figure 1 illustrate equally the effects of a carbohydrate meal. Details of the experiments are superfluous. There was no digestive leucocytosis.

A second type of experiment may be used to test the validity of the concept of digestive leucocytosis. The same criterion is used, viz., a change of the basal leucocyte count. Comparison is made of the basal

level of a given individual who has arrived at the laboratory in one instance without food and on the succeeding day having breakfasted. It has been found that the basal count of an individual who maintains a fairly regular regime will remain at a remarkably constant level day after day over a long period. The counts in these cases were made preferably in the early morning or fore-noon and with the assurance that there has been no infection, severe fatigue, loss of sleep or sustained emotional disturbance, otherwise variations such as those reported by Stetson (8), may creep into the picture. Such individuals appeared in the laboratory without breakfast (fig. 2, A) and on alternate days immediately after breakfasting. The basal level reached after an hour of recumbency was the same, irrespective of food intake on all days—allowance being made for a variation of three hundred cells either side of a mean, this variation being sometimes in the

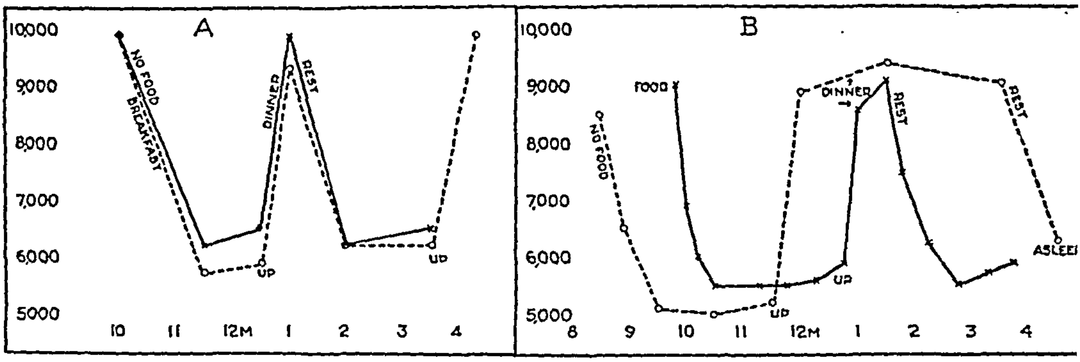

Fig. 2. A: Two leucocyte counts, made on successive days, on the same individual. Breakfast did not affect the drop to the basal level of recumbent rest; likewise a mid-day meal had no effect on the subsequent basal counts. B: Two individuals, one of whom had breakfasted; the basal leucocyte count was not affected by either the morning or noon-time meals. The changes recorded were due solely to posture and activity.

direction of increase in the count, equally often of decrease. Our records indicate this result upon eleven individuals in eighteen experiments. The average American breakfast therefore did not tend to increase the basal leucocyte count when precautions were taken to eliminate other causes of physiological leucocytosis.

The reaction to meals later in the day showed no deviation from the above findings—when two individuals, only one having breakfasted, appeared in the laboratory. There was a commensurate fall in the leucocyte count upon lying down until the individual basal levels were reached. Upon rising and going to a mid-day dinner the leucocyte count rose immediately to the activity level (fig. 2, B), but remained up only during the period of activity and fell according to rule upon lying down after the meal,

the rate and extent of the fall being independent of the preceding meals, although it should be noted in passing that the average afternoon basal count is sometimes slightly above that early in the day, a phenomenon which however is independent of meals and is irrelevant to this presentation. All of the evidence cited above indicates the absence of a true digestive leucocytosis; there is certainly no persistent rise of the basal count during the absorptive stage.

Since we had previously noted that localized peripheral stimuli acting reflexly may cause a transient rise in the leucocyte count, apparently by a vascular reaction, five series of counts were made with a view to determining the effects of gastric filling and temperature changes. Resting subjects with a basal count were given lemonade at different temperatures—approximately 5°C., 50°C. and 20°C., respectively. In each instance the subject drank to satiation—approximately three glasses. Counts made three minutes later showed a rise in every case, amounting with the cold solutions to a 15 per cent-30 per cent rise above the basal level. In the case of the hot drink the rise was higher, amounting to 22 per cent to 42 per cent of the basal count. In one instance the count was higher than the activity level, in all other cases slightly lower. It was further noted that within ten minutes the count was gradually falling so that the basal level was restored within half an hour. Water will cause the same reactions which seem to be related to the vascular alterations due to temperature changes and to the reflexes instituted by the acute gastric distention. Slow sipping of 500 cc. of water at room temperature caused no change in the leucocyte count. On the other hand a very small drink of water at body temperature induced nausea on three occasions, each accompanied by a sharp increase in the leucocyte count which is attributable to the vascular changes resulting from the emotional upset.

Animal experiments were resorted to in order to clarify the situation relative to gastric temperature and filling. Dogs were anesthetized with sodium barbital administered in solution through a stomach tube. The tube was later introduced and left in place to avoid the effects of repeated removal and reintroduction. The counts were made on blood drawn by deep puncture wounds of the upper lip, and checked with counts made on blood drawn from large veins. It should be stated that the counts on such animals remain constant for many hours, furthermore that spinal and medullary reflexes are extremely sharp and easily elicited (9). The results of the experiments are given in table 1 and may be summarized as follows: Rapid distention of the dog's stomach (350 cc.) by air or water at body temperature causes an instantaneous rise in the leucocyte count with a return to the initial level within a half hour. Similar quantities of fluid introduced at a slow rate do not affect the count. Either hot or cold water suddenly introduced have a similar but greater effect, the leucocy-

tosis persisting somewhat longer after the cold administrations. The promptness and transiency of these reactions place them definitely into the category of nervous reflexes affecting the vascular area (7, 9), and producing a sudden redistribution of leucocytes, with more gradual readjustment to normality. The rôle of the sympathetic nervous system has already been demonstrated by the authors (10).

Identical reactions of human subjects emphasize the limitations of the rôle which prandial libations can play in causing a rise in the leucocyte count. It is to be noted however that at meals fluids are usually sipped,

TABLE 1

DOGS ANESTHETIZED WITH SODIUM BARBITAL BY STOMACH	TEMPERATURE	INITIAL LEUCOCYTE COUNT*	LEUCOCYTE COUNTS MADE AFTER TREATMENT (MINUTES)								REMARKS
			3	10	15	20	30	40	60	90	
	°C.										
I. 150 cc. water. Introduced rapidly by stomach tube	50	9,400	11,120		10,000		9,840				
	8	10,280	12,200		11,400		10,680				
	50	16,800	18,240		17,000		16,640				
	40	16,800	17,600	17,120			17,000				Distention only
	5	13,000	14,600	15,000		14,450	14,000				
	40	13,120	14,520	13,480		13,000					Distention only
	5	11,680	13,000	13,400		12,800	12,120	11,800			
	40	12,000	13,600	12,260		11,650					Distention only
	4	15,600	16,880	18,400		18,800	16,800	16,000	15,500		Slow return to basal level
	50	15,260	17,200	16,680		16,000	15,750				
II. Air 300 cc. Rapid distention of stomach		13,600	16,600	14,980		14,400	13,280				
		13,120	15,200	14,000		13,420					
		10,800	13,600	12,640		12,000	11,120				
									Flow stopped		
III. Water (350 cc.) slowly introduced during 45 minutes	40	10,000	9,860		9,600		9,820	9,500	9,800	9,460	Slight decrease
	40	10,120	9,580		9,280		9,600	9,480	9,780	10,000	Slight decrease
	40	8,000	7,860		8,400		8,000	8,260	8,120		Slight decrease
	55	7,600	7,920		8,000		8,180	8,300	7,980		No change
	55	12,800	13,000		12,860		12,920	12,780	12,700		No change

* Initial counts made about two hours after anesthetic was given. The high initial counts are abnormal but immaterial to this investigation.

not guzzled, that the reactions described are immediate and are recovered from within the usual meal period. Furthermore the reactions are independent of nutriment and therefore can have nothing in common with our usual concept of digestive leucocytosis, a condition which our experiences indicate to be wholly fictitious.

The failure of other experimenters to obtain similar results may be attributed to failure to recognize the necessity of eliminating all variables except the one under investigation and to compare all counts with a basal norm established by a condition of absolute rest.

SUMMARY

The low *basal* leucocyte count of the resting state is wholly unaffected by the intake of large quantities of either protein or carbohydrate.

Sudden distention of the human stomach, or abrupt changes in gastric temperature, due to hot or cold fluids, cause an immediate, but mild and transient rise in the leucocyte count. Animal experiments indicate that these reactions are due solely to reflex vascular disturbances, but have no relation to the absorption of digest.

All experiments indicate that there is no digestive leucocytosis in normal adults.

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THE EFFECT OF CAFFEINE ON THE PREVAGUS AND POST-VAGUS CHICK HEARTS

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There seems to be a prevailing opinion among pharmacologists and physiologists that caffeine affects the force and frequency of the heart beat and that this effect is the result of the direct action of the drug on the cardiac muscle fibers. The work reported by Pickering (1) on Daphnia, Pitcher (2) and Heathcote (3) on mammalian hearts, indicates that dilute solutions of caffeine increase the rate and force of the heart beat while concentrated solutions decrease the frequency and amplitude of the beat. The general concept of the effect of caffeine on the cardiac muscle seems to have originated from the work of Pickering in 1893 (4). Pickering studied the effect of caffeine on the rate and rhythm of the cardiac contraction of 60 to 75 hour chick embryos. He was, therefore, studying the action of the drug on the heart before the entrance of nerves and apparently did not study the effect of caffeine on the innervated or neurogenic heart. Since the results of Pickering's experiments have been rather generally accepted without question and have remained unchallenged, it is deemed advisable to report his experiments, at this time, in some detail.

Pickering reported in his table on page 397, that dosages of caffeine equal to 0.00015 gram in one cubic centimeter of physiological saline solution (approximately equal to M/1400) increased the frequency of the heart rate from 88 to 100 per minute. Additional dosages of the same concentration applied to the same heart showed no further effect. More concentrated solutions, 0.0003 gram per cubic centimeter, reduced the rhythm of the same heart to 76 beats per minute: a dosage of 0.00075 gram proved fatal. In a second experiment, using a fresh heart, Pickering found that caffeine in a concentration equal to 0.0025 gram in a cubic centimeter of saline solution (approximately equal to M/100) produced a slight irregularity in the rhythm and the rate became much slower. He does not differentiate between the different chambers of the heart in these experiments, although he does make the following statement on page 384, "as I hope to show in the following pages that there are considerable differences in the attributes of the auricular contractile tissue and the ventricular tissue in the three day old chick embryo, it is important to bear in mind

that in my experiments the whole heart is under observation." The only place where Pickering mentioned the portion of the heart affected is in the last remark in the table where he states that "The auricular half of the heart gave two strong beats, the whole heart then passed into strong contraction and was irresponsive to stimuli." From these results Pickering concluded that caffeine affects the cardiac muscle directly and the nervous elements play only a secondary part. Since Pickering's experiments do not seem to be extensive enough to warrant his conclusions and as no experiments were conducted on the innervated heart, it was deemed advisable to study the effect of caffeine on the prevagus and postvagus hearts of chick embryos in order to ascertain whether the drug affects the rate and rhythm of the heart beat by its action on the cardiac muscles or on the nervous system.

Method and procedure. Fertilized chick eggs (pure blooded white leghorns) were obtained from the Poultry Department of the State College and incubated at 37 degrees in an electric oven. The eggs were gathered from the hennery every hour and a half, therefore they were in about the same stage of development at the time of incubation. The embryos were allowed to develop for the desired period of time and an opening an inch in diameter was made in the shell and the eggs placed in an observation chamber where the hearts of the embryos could be observed. The observation chamber consisted of a small electric oven placed on its back so that the embryo could be observed through the glass door of the oven by the aid of a binocular microscope. The temperature of the observation chamber was maintained at the same degree as the incubator. After the embryos had recovered from exposure during the opening of the eggs and the heart rhythm became uniform, the time required for fifty cardiac contractions was taken with a stop watch. One-half of a cubic centimeter of caffeine (Merck U. S. P.) solution, made up in physiological salt solution and warmed to 37 degrees was placed in the opening in the egg shell directly above the embryo. In the earlier experiments the membranes surrounding the yolk of the egg were cut to allow caffeine to diffuse more quickly into the embryo but in later experiments this was found to be unnecessary and was discontinued. At various intervals after the addition of the drug the rate of heart beat was determined, as before, by taking the time for fifty cardiac contractions.

In the chick embryo there is no innervation of the heart for the first five or six days after incubation, therefore the heart beats without extrinsic nerves for the first 120 hours of incubation. After the vagus completes its connection with the heart, the frequency of the beat is presumably under the control of the nervous system. It is possible, therefore, to study the effect of caffeine upon the nerveless and innervated heart and in this way to determine if the drug affects the contractility, irritability or conduc-

tivity of the cardiac muscle or the frequency or amplitude of the beat by virtue of its action on the nervous system.

RESULTS. Eggs were incubated from 48 hours to ten days and the effect of caffeine solution on the embryonic heart was determined by noting whether or not any difference occurred after the addition of caffeine in the rate of contraction, force of heart beat or conductivity of the impulse. The addition of M/100 caffeine to the prevagus heart produced a decided irregularity in the auricular-ventricular rhythm. The auricles usually continue to beat at a regular and definite rate but the rhythm of the ventricular contraction is much slower. The ratio of the auricular to the ventricular beat may vary from 1–1 to 5–1. At other times the ventricular may beat at a reduced rate which is quite independent of the auricles. This condition approaches what is known as heart block in mammals. Gradually the entire heart is brought to a standstill in the diastolic condition. The cardiac muscle, however, still remains irritable and responds by contraction to mechanical and electrical stimulation.

The excised heart behaves in all respects the same as the intact heart in the embryo. The excised heart, after it has come to a standstill in caffeine solution, which may require an exposure of an hour or more, will remain irritable to electrical stimulation for several hours. If the excised heart is severed at the auricular-ventricular junction, the auricles will continue to beat for an hour or more while the ventricles soon come to rest in the diastolic phase. If the quiescent chambers are transferred to physiological saline solution, cardiac contractions are resumed within two or three minutes. Upon retransferring to the caffeinated solution the contractions soon cease.

The behavior of the prevagus heart treated with dilute solution of caffeine, M/1,000 or M/10,000 is different in some respects than when treated with stronger solution. The rate of the beat is slightly accelerated for a few seconds after the addition of the drug, followed by a gradual return to normal and after an exposure of several minutes the contractions of the ventricle become decidedly irregular while the auricles continue to beat at a normal and regular rate.

In the case of the older embryos there is a different situation. The embryonic heart is innervated by the vagus on the fifth or sixth day (Lillie). Therefore, in the older embryos the heart beat becomes somewhat complicated by the controlling action of the nervous system. The results obtained from the action of caffeine on the postvagus or innervated heart are different in degree from those described on the prevagus heart. Caffeine in the foregoing concentrations produce an initial stimulation which is quickly followed by an irregularity in the rhythm and a marked reduction in the rate of contractions of the entire heart. The frequency of the beat of the auricles as well as the ventricles becomes very slow with frequent

long pauses. During the period of inactivity the heart always remains in the diastolic condition and is sensitive to external stimulation. If the heart, stopped in caffeine solution, is removed from the embryo and kept in the same solution, the auricles will usually resume beating but ventricles seldom recover. If the excised heart is severed at the auricular-ventricular junction and the chambers placed in caffeine solution the auricles may continue to beat for two or three hours but the ventricles stop in diastole within a few minutes. They remain, however, irritable to external stimulation.

CONCLUSIONS. It is apparent from the foregoing results that the behavior of the prevagus heart is somewhat different than the postvagus when treated with caffeine solution. In the former condition the rate of the auricular beat is not significantly affected by the dilute solutions of the drug: the ventricles, however, show a decided irregularity and a marked reduction in the rate of pulsations. In the prevagus heart the beats are supposed to be the result of rhythmic contractions of the cardiac muscle fibers. The contraction wave originates in the sino-auricular node and is conducted through a system of modified muscle fibers, auricular-ventricular bundle, to the other chambers of the heart. If the conductivity of the fibers composing the auricular-ventricular bundle is decreased, the impulses arising in the sino-auricular node are completely or partially prevented from reaching the ventricles, therefore the latter chamber beats slowly or independent of the auricles. *Any factor that influences the* auricular-ventricular rhythm may be considered to affect the conductivity of the modified muscle fibers. This seems to be the case with caffeine.

In the case of the innervated heart the entire organ is more or less under the control of the nervous system and the entire heart shows the effect of the drug by an irregularity of the rhythm and a reduction in the rate, which is probably due to stimulation of the vagus nerve. It seems apparent, therefore, that the effect of caffeine may be twofold; 1, reduction in the conducting power of the muscles composing the auricular-ventricular bundle of His, and 2, stimulation of the vagus supplying the innervated heart. The drug does not seem to produce tetany in the cardiac muscle as is evident by the fact that the heart will respond by contracting after it has been brought to a standstill in caffeine solution.

SUMMARY

Prevagus and postvagus hearts of chick embryos were treated with M/100, M/1,000 and M/10,000 caffeine solution in order to determine if the drug affects the cardiac muscle or the nerves supplying the heart.

The results of a series of experiments indicate that caffeine in solution may destroy partially or completely the conducting power of the muscle fibers composing the auricular-ventricular bundle. It also depresses the rate of contraction of the innervated heart through its action on the vagus

nerves. Caffeine, in the concentrations used, does not produce tetany and apparently does not prevent contraction in the cardiac muscle.

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STUDIES IN THE MOTOR ACTIVITY OF THE LARGE INTESTINE

III. The Longitudinal and Circular Activity of the Distal Colon

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In an earlier report (1) we described a type of motor activity characteristic of the distal colon of the dog, which was recorded by means of serial balloons as simultaneous pressure changes over long segments. This activity was most marked in the region of the internal anal sphincter, and was usually the only activity appearing in the distal colon. Occasionally the entire colon pulsated rhythmically throughout the course of long tracings, but the usual finding was anally-propagated activity in the proximal portion, with a reciprocal relationship to the rhythmic pulsations of the distal. It was assumed that the Pendelbewegungen described by the early German workers, and analyzed by Magnus (2) as longitudinal, might be responsible for such synchronous pressure changes over long segments. Other possible mechanisms which suggested themselves were rhythmic segmentation or haustral segmentation under the influence of a coördinating apparatus, or some muscular function peculiar to the colon.

This study is an attempt to separate the longitudinal from the circular activity of the distal colon, as a basis for further work on the nature of the rhythmic pulsations. It was necessary to select a method which would enable us separately to record changes in length and in the short diameter, and which could be used on unanesthetized dogs. An apparatus was constructed for this purpose (3), which consisted essentially of a movable plunger which could be inserted into a closed intestinal pouch, the plunger moving in a protecting tube to which tandem balloons were attached as previously described. Records were taken from water manometers of the pressure changes in the balloons. A similar pressure-volume record was obtained from movements of the plunger against a balloon, filled with air, and enclosed in a cylinder which was clamped outside the pouch.

The colon was transected in dogs which had previously been cecostomized for a balloon study of the intact colon. The left colic vessels were

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used as a landmark, the transection being made at different levels from 3 to 15 cm. above the point of entry of these vessels in the distal colon. The distal stump was invaginated and closed off to form a pouch opening by anus, and the proximal stump was sutured into the abdominal wound as a colostomy. There was considerable post-operative hemorrhage from the anus, and in one case complete prolapse of the pouch with subsequent death of the animal. In those dogs which survived the immediate effects of the operation, however, there was no significant loss of weight on the ordinary laboratory diet. One animal is still in use 13 months after operation.

The results reported below were obtained on unanesthetized animals, sufficient time, usually two weeks, being allowed for recovery from the operation. The dogs were trained to lie quietly on comfortable pads. The apparatus was inserted through the anus, and the plunger extended beyond its tube, against the end of the pouch, by inflating the balloon in the cylinder. The pressure in this balloon was usually about 10 cm. water with the apparatus adjusted and clamped in place. Inflation of the tandem balloons was carried out as in our previous work. Balloon tracings were also taken from the proximal segment at the same time with the distal record. In addition, six-balloon tracings were made of the entire colon, following transection, for comparison with records on the same dogs with intact colon.

In the following we have applied the term peristaltic, for convenience, to types of activity which appear on our balloon records as anally-delayed contractions (1).

Balloon tracings of the entire colon after transection in the region of the splenic flexure showed little alteration in the activity of either segment or in the reciprocal relationship between them (fig. 1). With the incidence of peristalsis in the proximal segment, the rhythmic pulsations of the distal segment were depressed. The rhythmic pulsations reappeared with the termination of the proximal type of activity, and often replaced peristalsis in the proximal segment. In only one dog, whose distal segment was so long as to include a portion of the proximal colon, did we observe spontaneous peristalsis in the distal segment (fig. 6). After transection, in the other animals, separation between proximal and distal types of activity occurred sharply at the site of transection. As in the intact colon, it was noted that a reciprocal relationship sometimes existed between distal activity and activity in the proximal colon which could not be classified from the record as peristaltic. In these tracings, however, many of the proximal periods of activity were unquestionably peristaltic. On the other hand, where only rhythmic pulsations appeared in the proximal as well as the distal colon, no proximal-distal reciprocity could be observed.

Records of the longitudinal and circular activity of the distal segment were found to vary with the length of the pouch, but were qualitatively

consistent in the dogs studied. The longitudinal tracings showed a marked periodicity, not unlike the periodicity noted in proximal balloon tracings. Unlike balloon tracings of the distal colon, longitudinal periods were frequently accompanied by high, lasting tone changes. The two types of contractions observed on balloon records were also seen on the longitudinal record, with similar rhythm. In most tracings, the uppermost balloon showed less activity than the lower, which was usually in the region of the internal anal sphincter (fig. 2). In many tracings only the plunger and the sphincter balloon showed activity, intervening segments remaining quiet. An invariable observation has been that the regular rhythmic type II activity of the balloons is depressed during activity of the plunger, sometimes becoming completely quiet (fig. 3), and sometimes, with high plunger activity, being replaced by an irregular type I activity on high tone (fig. 4), coincident with the peaks of the longitudinal tone changes. With cessation of plunger activity, there is recovery of the rhythmic type II contractions in the balloons. In one dog with a short distal segment (transection just above the left colic vessels) tracings usually showed no

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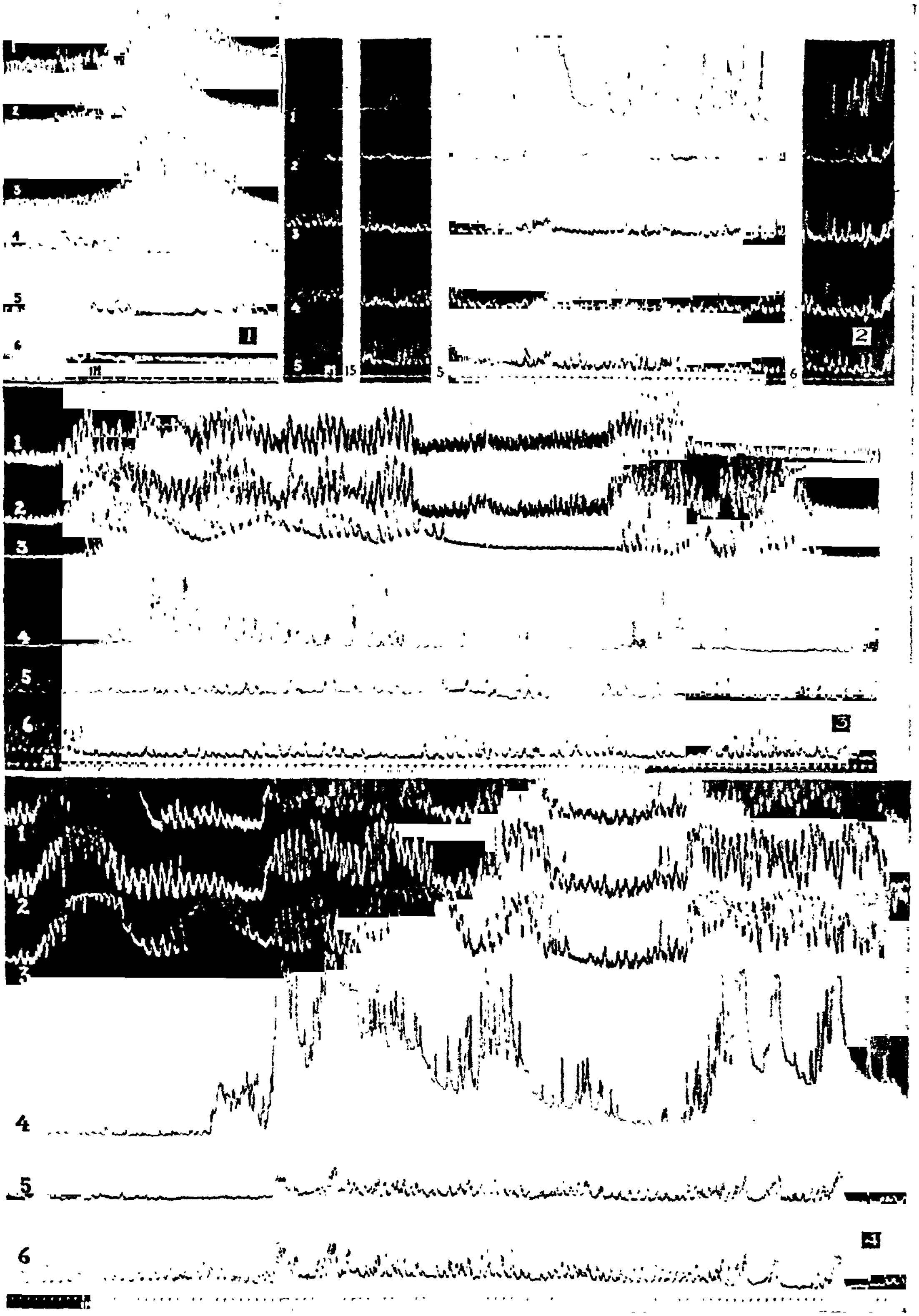
Fig. 1. Six-balloon tracing of entire colon after transection just above left colic vessels. Balloons 1 to 3 are in proximal segment, 1 at level of ileo-colic junction. Distance between balloons approximately 5 cm. Balloons 4 to 6 are in distal segment, 6 at level of internal anal sphincter. Interval between balloons is approximately 3 cm. Time tracing in minutes.

Fig. 2. Distal colon activity as recorded by plunger-balloon apparatus. Transection 10 cm. above left colic vessels. 1 is manometer record of plunger movements. 2 is balloon at upper end of pouch, attached to extended end of plunger, and moving with the latter. 3 to 5 are fixed tandem balloons on plunger apparatus, 5 at level of internal anal sphincter. Interval between tandem balloons 3 cm. Time tracing in minutes. Rythmic pulsations progressively increased toward lower end of pouch, depressed during plunger activity except at peaks of plunger tone, when balloons show tone rise with irregular type I contractions. No evidence of peristalsis.

Fig. 3. 1 to 3 are tandem balloons in proximal segment. 4 is plunger, 5 and 6, balloons in distal segment. Colon transected 10 cm. above left colic vessels. Periods of plunger activity coincide with periods of peristalsis in proximal segment, and with depression of distal balloons. Distal balloons (5 and 6) show augmented activity during quiescence of plunger and proximal segment, except that during last period of proximal activity, appearing on balloons 2 and 3 only, not peristaltic, balloon 6 is augmented. Time tracing in minutes.

Fig. 4. Preparation and apparatus same as in figure 3. High plunger activity coincident with peristalsis in proximal segment. Distal balloons are not depressed during plunger activity, but the regular type II contractions on balloon 6 are replaced by irregular type I contractions on high tone during peaks of plunger tone changes. During high plunger tone the animal whined as if in pain, the tail was lifted, and the hind legs brought forward as in defecation. These movements usually coincided with the tone changes in the distal balloons, but not invariably. This figure was taken from a 6 hour tracing, in which this picture was periodically repeated. Time tracing in minutes.



Figs. 1 to 4

activity except in the plunger (fig. 5A). During longitudinal tone rises there was a slight depression of balloon tone, followed by a rise nearly simultaneous with the peak of the plunger tone change. Rarely, in this dog, the balloons also showed periods of activity, which occurred during the longitudinal period in such manner as to give the appearance of splitting the latter (fig. 5B). Following a period of general inactivity, two longitudinal periods would occur, with a brief intervening period in which the balloons alone were active.

The longest distal segment studied was one in which the transection was made about 15 cm. above the left colic vessels. In tracings from this

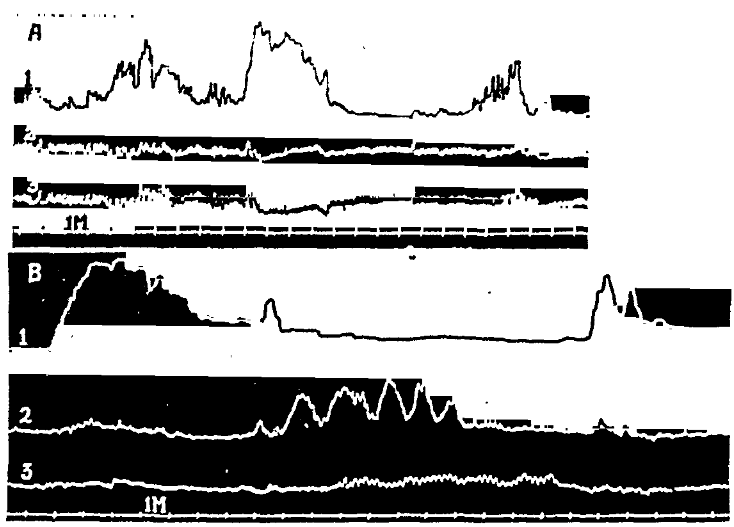


Fig. 5. Distal colon activity as recorded by plunger-balloon apparatus. Transection just above left colic vessels. 1 is manometer record of plunger movements. 2 and 3 are tandem balloons, 3 at level of internal anal sphincter. In A the plunger alone is active, balloon tone being slightly depressed during the rise of plunger tone. In B the balloons are also active, and periods of balloon activity bear a reciprocal relationship to periods of plunger activity. Time tracing in minutes.

animal, the appearance and relationships of the plunger record were quite similar to balloon records taken from the same dog's proximal colon before transection. Plunger activity occasionally preceded activity on the balloons below in such manner as to place the plunger tracing peristaltically into serial relation to the balloon records (fig. 6). When rhythmic pulsations were initiated, there was depression of the plunger.

The possibility, suggested in this observation, that movements of the plunger were produced by peristalsis sweeping the easily movable plunger periodically toward the anus, led to various modifications of the apparatus. The end of the plunger is capped with a metal ball. Reducing the size of this was found not to reduce either the amplitude or the rate of plunger

movements. Protecting the extended end of the plunger with a coil spring of such strength that, when the recording balloon was inflated against the plunger, the spring could be grasped tightly and compressed downward without moving the plunger, did not alter the plunger record.

In acute experiments (8) the belly was opened, the plunger apparatus inserted by anus, and the free end of the plunger stitched in place through the wall of the colon. These records show the same type of longitudinal activity as that recorded on unanesthetized dogs.

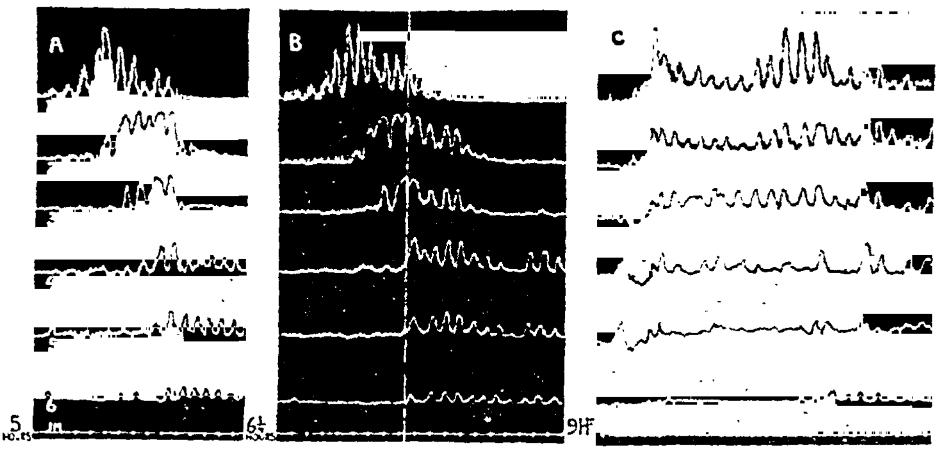


Fig. 6. Distal colon activity as recorded by plunger-balloon apparatus. Colon transected 15 cm. above left colic vessels. Representative periods of activity in a 12 hour tracing. Hours from beginning of tracing indicated between periods. Time tracing in minutes. 1 is manometer record of plunger movements. 2 to 6 are adjacent tandem balloons, 2 about 5 cm. below end of plunger, 6 at level of internal anal sphincter. In A plunger activity precedes and accompanies a period of peristalsis which can be traced as low as balloon 5. At this point rhythmic pulsations appear simultaneously on balloons 4 to 6, with depression of peristalsis and depression of plunger activity. In B peristalsis can be traced as low as balloon 4, at which time rhythmic pulsations are initiated throughout the segment, with depression of the plunger. Transition from peristalsis to rhythmic pulsations indicated by vertical line. In C there is an irregular, non-periodic activity, with failure of circular-longitudinal reciprocity.

As additional evidence that the plunger movements were not produced by the translational effects of peristalsis, we have obtained records from a small balloon tied on the end of the plunger, moving with the latter, and led out to a recording manometer by substituting for the plunger a small-bore metal tube with side-arm and flexible manometer connections projecting through the open portion of the plunger apparatus. Such tracings show typical plunger activity, without any evidence of peristalsis, the balloon at the extreme upper end of the pouch recording the same type of activity as is seen on the lower balloons, and following the other balloons in its periodic changes more closely than it follows the plunger (fig. 2).

Usually for a period of one hour or more following the insertion of the apparatus, the only activity recorded by the plunger is a gradual fall in tone. The balloons, during this period, are very active. At the time when the tone of the plunger tracing has fallen to a constant level, the balloon activity is usually much reduced, and it is at this time that periodic plunger activity appears. Assuming that the possibility of mechanical error in this portion of the tracings, such as inability to extend the plunger to the end of the pouch due to the demonstrably high circular tone and activity, might invalidate that portion of the record, we have discarded the first portion of each tracing in presenting these observations, and have continued the tracings until constant tone levels were obtained.

Balloon records from the proximal segment taken simultaneously with the records from the distal colon, showed, in general, that periods of plunger activity in the distal segment usually coincided with periods of proximal activity, if the latter were peristaltic. Usually the distal plunger tracing showed augmentation, and the distal balloons depression, slightly in advance of the proximal period (figs. 3, 4). The recovery of rhythmical distal balloon activity was associated with quiescence in the plunger, and either quiescence or rhythmic pulsations in the proximal segment. In one dog rhythmic pulsations were the only type of activity usually found in the proximal segment. We were unable, in tracings of this type, to establish any constant relationship between either the longitudinal or circular activity of the distal segment, and the proximal activity, although the longitudinal-circular reciprocity in the distal segment was constant. In tracings from the same animal, however, in which the more usual, peristaltic type of proximal activity was observed, longitudinal activity in the distal segment was more pronounced, distal longitudinal periods coincided with the proximal periods, and were associated with depression in distal balloon activity as described above (fig. 4). Often in all the animals studied, at the peaks of very high longitudinal tone changes, the tail would be lifted and the hind legs brought forward as in defecation. Occasionally the animal would whine as if in pain during these periods.

Using depression of proximal activity as an index (4) we compared the stimulating effect of the insertion and presence of the plunger apparatus, with that of the flexible balloon systems previously used. Such comparisons, made on two dogs, showed that in both cases the plunger apparatus produced longer and more complete depression than the flexible systems. In addition, it was noted that the insertion of the flexible systems into the distal pouch was followed by proximal depressions lasting as long as 75 minutes, as compared with a maximum of approximately 30 minutes in the intact colon. For some time after return of activity in the balloon nearest the cecum, the second and third proximal balloons were not fully recovered.

Learmonth and Markowitz in 1930 demonstrated the presence of inhibitor fibres to the distal colon of the dog in the lumbar colon nerves which reach the distal colon with the inferior mesenteric artery. (5) Our inability to find any gross alteration of the proximal segment after transection above this point might indicate that the distribution of such inhibitory fibres is largely distal to the cut. On the other hand, although the mesocolon was sectioned in our dogs to its root, we do not know what fibres from the inferior mesenteric ganglion reached the proximal colon through the root of the mesocolon. These workers stated, in addition, that motor fibres from the pelvic nerves ascended in the wall of the colon itself. We did not find a decrease in the activity of the proximal segment after transection.

Although the spontaneous motility of neither segment was altered after transection, our observations do indicate an increase in the irritability of the distal segment. Whether we are dealing here with an altered nervous control, or with some sort of adaptation following the transection and colostomy, our work does not show.

The reciprocity which we noted between typical proximal activity and typical distal activity was not diminished after transection. A purely mechanical basis, such as transfer of material, or the pull of one segment on another during activity, is thus excluded from the proximal-distal reciprocity, and the anatomical continuity of the nervous apparatus in the wall of the colon is shown not to be essential. The fact that Pearcy and Van Liere in 1926 (6) made similar observations in their study of the inhibiting effect of distention of any segment on the activity of other segments of the gastro-intestinal tract may be significant.

The assumption that a plunger such as we employed in the distal segment would not be moved by circular activity is probably unwarranted. New in 1899 (7) made this assumption in suggesting an "apparatus to show longitudinal movements of the intestine." His device consisted of two glass tubes, the one sliding within the other, producing pressure-volume changes on the contained air which were recorded by a tambour. In our apparatus we were able, by means of the tandem balloons, to determine whether or not circular activity appeared in the segment during movements of the plunger. In the isolated instance mentioned above in which balloon records showed peristalsis in the upper portion of the pouch accompanying plunger activity, the latter might be interpreted as simple translation toward the anus by the circular waves. This possibility is excluded, however, in all other cases by the absence of any such circular activity during plunger movements, and especially by finding in acute experiments (8) similar movements of the plunger during peristalsis, where translation is ruled out by sewing the end of the plunger into the gut wall. In our apparatus the size of the plunger was reduced to a minimum, about 2 mm.

in diameter of cross section. Reducing the size of the ball which capped the plunger, so as to afford less opportunity for waves of constriction to sweep it downward, and protecting the exposed end of the plunger with a spring whose coils would be moved by such waves, leaving the plunger unaffected, did not alter the record. It is doubtful if either of these modifications of the apparatus would completely protect the plunger from the influence of circular waves; it is significant, however, that they had no effect in reducing plunger movements.

A purely artificial basis for the circular-longitudinal reciprocity recorded by this apparatus is to be considered. It is possible that high circular activity in the region of the extended portion of the plunger might immobilize the latter, allowing plunger movements to occur only during circular inactivity. That this is not the explanation is shown by the fact that the upper end of the pouch is comparatively quiet, the circular activity being greater and greater as the sphincter region is approached. It is the latter region which reciprocates most strikingly with the plunger, a region in which circular activity could not affect movements of the plunger which here lies within its rigid protecting tube.

The relationship which we have recorded between the circular and the longitudinal activity of the dog's distal colon indicates a reciprocity which is not so invariable as would be predicted from the frequently quoted generalization of Exner (9): "that in a freely moving tube which contains as integral parts of its wall longitudinal and circular muscle, contraction of the former must bring about shortening of the tube and widening of its lumen, contraction of the latter lengthening of the tube and narrowing of its lumen." In 1883 Fellner (10) failed to confirm Exner's law in its entirety, on the distal colon of the dog, finding longitudinal relaxation frequently unaccompanied by circular contraction. He sectioned the dog's rectum, in acute experiments, attached the distal stump to a writing lever, and took simultaneous records from it and a balloon within the lumen. From his observations on the effects of nerve stimulation, he deduced a reciprocal innervation for the longitudinal and circular coats; the nervi erigentes supplying motor fibres to the longitudinal and inhibitory to the circular, the hypogastric motor to the circular and inhibitory to the longitudinal. Elliott and Smith, however, in 1904 (11) showed that stimulation of the pelvic visceral nerves in the dog had the same effect on both the longitudinal and the circular coat throughout the colon, shortening preceding constriction by about 30 seconds. Carlson (12) has reported that stimulation of the peripheral end of the sectioned hypogastric reverses the state of the distal colon, producing activity if the gut is quiet, and quiescence if it is active.

In the dog's distal colon we have seen the circular-longitudinal reci-

procity persisting for hours, only to be replaced by activity in which there is depression of the rhythmic circular type II activity during longitudinal periods, but no diminution in circular tone or type I contractions. This variability of the relationship between longitudinal and circular activity would indicate that the purely mechanical basis for such reciprocity suggested by Exner is not of prime importance in this region of the gut.

Luderlitz (13), Biedermann (14), Trendelenburg (15), and others have observed a longitudinal activity during peristalsis. Biedermann confirmed Exner's law in his work on the earthworm, stating that longitudinal contractions always preceded circular; Luderlitz saw shortening of the rabbit's small intestine as the first response to distention, with circular contractions much later; Trendelenburg found that during peristalsis there was a longitudinal contraction for every circular, preceding the circular contraction by about $\frac{1}{4}$ to $\frac{1}{2}$ wave length. Leusden and Riesser have recently criticized Trendelenburg's conclusions on the basis of their observation that the longitudinal-circular reciprocity could be altered at will by changing the pressure in the segment (16).

Our work indicates that there is a shortening of the distal colon during proximal peristalsis. Further, the shortenings of the distal segment stand in the same reciprocal relationship to the rhythmic pulsations of the distal colon as does proximal peristalsis. It is suggested that such longitudinal activity in the distal segment is the longitudinal component of proximal peristalsis, usually the only component which appears in the distal colon. In the intact colon we have reported a shifting point of separation between the proximal and the distal types of activity, peristalsis sometimes traversing the entire colon. After transection we observed the circular phase of peristalsis below the transection in only one dog, in which the transection was so high as to include most of the colon in the distal segment. In the other animals, with somewhat shorter distal segments, the transection, wherever it happened to be made, was the boundary between proximal and distal types of balloon activity. It would appear from these observations that either the transection itself, or the altered state of the empty distal colon after colostomy, interrupts the propagation of the circular phase of peristalsis more completely than that of the longitudinal phase.

Langley and Orbelli in 1911 (17) stated that the dragging down of the cloaca in the frog on stimulation of the sacral autonomic nerves was primarily due to contraction of special bands of muscle corresponding to the recto-coccygeal muscles of the mammal. We have not investigated the possible activity of such muscles in the dog. Observations of the perineum during periods of longitudinal activity have not given any indication that accessory muscles were active unless defecation movements were obtained. Our longitudinal tracings are strikingly similar to those presented by

Fellner (10) in which curare was given in amounts sufficient to paralyze the respiratory muscles, and in which he assumed that the activity of such muscles was ruled out.

The stationary contractions of the dog's distal colon which we have termed rhythmic pulsations are shown by these observations to be due not to contractions of the longitudinal coat, which presumably would present the picture of synchrony over long segments, but to contractions of the circular coat unaccompanied by generalized shortening of the active segment.

The writers wish to acknowledge their indebtedness to Dr. A. J. Carlson, under whose direction this work was conducted.

SUMMARY

1. Transection of the dog's colon in the region of the splenic flexure does not produce marked alteration in the spontaneous motor activity of either segment.

2. The reciprocal relationship observed in the intact colon between the proximal and distal types of activity is preserved after transection.

3. Peristalsis as a wave of constriction does not pass from the proximal to the distal segment over a transection in the region of the splenic flexure.

4. The longitudinal activity of the distal colon bears a reciprocal relationship to the circular activity of that segment, the relationship being most marked in the anal sphincter region, which shows the greatest circular activity.

5. The longitudinal activity of the distal colon runs parallel to proximal peristalsis, if the latter is present in the proximal segment. With other types of activity in the proximal segment, the distal longitudinal activity shows no constant relationship.

6. The irritability of the distal colon is increased after transection and colostomy.

7. The rhythmic pulsations of the distal colon are constrictions unaccompanied by generalized shortening of the segment.

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AN ANALYSIS OF THE RHYTHMIC VASCULAR CHANGES IN THE UTERUS OF THE RABBIT

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A number of the characteristics of the rhythmic vascular changes in the uterus of the rabbit have already been described (Markee, 1929, 1932). This study deals with the factors that determine the duration of the vascular cycle, the phases of the vascular cycle, the effect of adrenalin on the cycle and the relation of the vascular to the muscular cycle.

I. FACTORS INFLUENCING THE LENGTH OF THE CYCLE. Since different transplants in the same eye have independent rhythms it seemed advisable to consider more fully the factors that might influence the duration of the cycle.

A. *Part of the uterus from which the transplants are taken.* In the intact uterine horn, the vasoconstrictions begin at the tubal end and pass down toward the cervix. A few seconds later vaso-dilatation begins at the cervical end of the uterine horn and passes towards the tube. This suggests that the tubal end may be a regulator or "pacemaker" for the vaso-constrictions. If the conditions in the endometrium are similar to those in cardiac musculature, one should expect transplants of endometrium from the tubal to have a shorter cycle than those from the cervical region if a regulator exists. Double transplants were hence made to the eyes of 12 rabbits, each receiving one piece from each end of the uterine horn but the difference in the duration of the cycles of the paired transplants from opposite ends was neither constant enough nor great enough to be decisive.

B. *Microscopic structure of the transplants.* Although the microscopic structure of the transplants is very similar to the structure of the endometrium in its normal position, there was a slight increase in the amount of fibrous connective tissue in all transplants examined. In general, those transplants that have the most noticeable increase in the amount of fibrous connective tissue have the longest cycles. It therefore seems probable that the increase in fibrous connective tissue may be correlated with the lengthening of the rhythm.

The arrangement of the blood vessels in the transplants has been studied

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by the injection method and by the examination of thick sections of uninjected material. There does not seem to be any significant alteration in the vascular patterns in endometrium transplanted to the iris. It was previously shown that some, if not all, of the functional vessels in the transplant were present in the uterus before implantation. However, it seems that the architecture of the vascular pattern may be altered somewhat when the iridial vessels anastomose with the cut ends of those in the transplant, and that this slight modification in the pattern may be another factor in the determination of the duration of the vascular cycle. There is considerable variation in the amount of time that elapses between transplantation and the re-establishment of circulation within the transplants. Those transplants that vascularize most rapidly have the shortest cycles and the greatest number of blood vessels per unit area, as established by observation in the living animal and by examination of stained sections. Although these three factors have been considered separately it is evident that their occurrence and their effect on the vascular rhythm are intimately related.

Since Hofbauer (1929) described myoneural bundles in the human uterus, it seemed possible that they are the ones that control the vascular rhythm. These elements, if present in the guinea pig and in the rabbit, are not grouped together in a well defined bundle as they are in man and it was not possible to show that they are causally related to the vascular rhythm either in the endometrium *in situ* or in the transplants.

C. *The local use of nicotine.* Since the wave of the vasoconstrictions passed down the uterus towards the cervix and the vasodilatation in the opposite direction, it seemed feasible to apply nicotine locally to prevent the passage of any nervous stimuli along the uterine horn. When a solution of nicotine is painted on the uterine horn near the middle the vasoconstrictions pass down to this area but not beyond it and none occur below it but the results were far from satisfactory because after a few minutes the vascular changes ceased. The vasodilatations seem to be the passive part of the vascular cycle for they will originate where the uterus is bathed in nicotine. The vaso-constrictions, on the contrary, will neither pass that point nor originate below it.

D. *Transection of a uterine horn.* In view of the fact that the vasoconstrictions would not pass the area painted with nicotine it seemed advisable to discover whether the upper and lower portions of the uterine horns had a common rhythm when divided from each other. Therefore, the uterine horn on one side was transected about half way between its two ends. Since such a trauma arrests the vascular changes for some time, the animals were examined three days later. At that time there was only a slight difference in the rhythm in the two parts, that in the upper segment being slightly shorter than that in the lower. The rhythm on the unoperated

side was slightly shorter than that in the upper segment on the operated side. Therefore, there is very little evidence in support of the hypothesis that a regulator exists in the tubal portion of the uterine horn.

II. THE PHASES IN THE VASCULAR RHYTHM. In addition to being modified by the time of the day and the amount of the follicular hormone present in the blood, the vascular cycle is also modified by fright. If a rabbit is frightened during the middle third of a vascular cycle, a vasoconstriction occurs 17 to 20 seconds later. Fright in any other part of the cycle does not modify either that or a subsequent cycle. Therefore, each vascular cycle may be divided into four periods or phases: a period of vasoconstriction (5 to 15 seconds); a refractory period (20 to 35 seconds); a resting period (10 to 30 seconds); and a latent period (17 to 20 seconds).

Fright was followed by vasoconstriction only during the resting phase. Since this period is more variable in length than the others, it largely determines the length of the individual cycles. Thus during the hours of the day when the cycles are longest, this period is greatly lengthened, and it is shortened proportionately during the hours of the day when the cycles are shortest. The period of vasoconstriction is the only other one the length of which is variable. By refractory period is meant the time during which vasoconstriction cannot be induced by fright. This period is constant in any given transplant but differs in length in different transplants and so do all the others. The latent period is the interval between the time that the animal is frightened and vasoconstriction.

A. *Multiple transplants.* That the "phase or period mechanism" lies within, rather than without, the uterus is indicated by the reactions of different transplants in the same eye to fright. Since different transplants to the same eye have independent rhythms, it is possible to frighten an animal when one transplant is in the resting and another in the refractory period. If this be done, vasoconstriction occurs only in the transplant that is in the resting period.

B. *Removal of the adrenals.* The latent period of 17 seconds is so long that it hardly seems that the stimulus for vasoconstriction can be a nervous reflex. Moreover, it has already been shown that stimulation of the cervical sympathetic does not modify the vascular cycle. In order to discover the effect of adrenalectomy on it, both of the adrenals were removed from 12 female rabbits having successful transplants.

It was not possible to induce vasoconstrictions in the transplants of these animals either immediately after the operation or later by frightening them. Only the rhythmic vascular changes persisted and the cycles were lengthened as the animals weakened.

C. *The latent period.* Stuart and Rogoff (1916) found that the time required for the blood to pass to the eye from the adrenals was several seconds less than the latent period of mydriasis produced by splanchnic stimulation.

They injected 1.5 to 2 cc. of a 1/50,000 solution of adrenalin with methlyn blue into the femoral vein and noted that mydriasis is nearly equal to the interval between the injection of adrenalin into the femoral vein and mydriasis. Although stimulation of the splanchnics and frightening the animal are very different, the above facts may, with a fair degree of safety, be regarded as indicating that adrenalin reaches the blood stream very soon after an animal is frightened. If that assumption is valid, only a very small part of the latent period of 17 to 20 seconds is accounted for by the time required for adrenalin to reach the blood stream after fright. Koppanyi (1930) found that the circulation time in the rabbit was about 6 seconds, and that the pulmonary circulation time was a little less than half of that. It therefore seems that 13 to 17 seconds elapse between the time that adrenalin reaches the eye and the appearance of vasoconstrictions in the uterine transplants, from fright.

Various amounts of adrenalin were injected intravenously (see table) and the latent period, the duration of the vasoconstrictions and the time of

TABLE 1

Effect of injection of adrenalin on blood vessels in uterine transplants (18 rabbits)

MGM. PER KILO	LATENT PERIOD	PERSISTENCE OF VASOCONSTRICTION	REAPPEARANCE OF CYCLE
0.00002 mgm.	17 seconds	8 seconds	75 seconds
0.0006 mgm.	17 seconds	14 seconds	75 seconds
0.001 mgm.	17 seconds	25 seconds	180 seconds
0.005 mgm.	9 seconds	140 seconds	12 minutes
0.01 mgm.	6 seconds	252 seconds	22 minutes

reappearance of the vascular cycle were noted. The length of time that the vasoconstrictions persisted increased as the amount of adrenalin injected was increased. However, the increase in the length of time that the vasoconstrictions lasted was not proportional when large amounts of adrenalin were injected. The length of time that the vasodilatation persisted was also roughly proportional to the length of the period of vasoconstriction. The ratio was about 6 to 1 as it is in the spontaneous vascular cycle. When adrenalin was injected slowly the effect on the vascular rhythm was proportional, not to the total amount injected, but to the amount injected within the first 15 seconds. It therefore seems that either in the normal animal the vessels of the uterus react only to the adrenalin that is liberated during the first 15 seconds after the animal is frightened or that the adrenals only secrete adrenalin for 15 seconds. Since fright may be very prolonged, the first alternative must be true and as a result this method can be of value only in a study of the amount of adrenalin produced during the first 15 seconds of fright.

If a rabbit is merely slightly startled during the resting period the subsequent vasoconstriction persists about 8 seconds, but if it is severely frightened the vasoconstriction persists for about 25 seconds. Since the injection of 0.0002 mgm. per kgm. causes a vasoconstriction that persists for 8 seconds and 0.001 mgm. a vasoconstriction that persists 25 seconds, these amounts may represent the range of secretion of adrenalin during the first 15 seconds of fright in the rabbit. The fact that the latent period of the injection of small amounts of adrenalin is the same as the latent period after frightening an animal also suggests that adrenalin reaches the blood stream almost immediately after a rabbit is frightened.

Injections of adrenalin were made in the different periods of the vascular cycle. It was found that the smaller amounts (0.0002 to 0.001 mgm.) would not induce a vasoconstriction if injected in the refractory period. However, the larger amounts (0.0005 to 0.01 mgm.) induced a vasoconstriction in any period of the vascular cycle. It therefore seems that amounts 5 times greater than those normally produced will "break through" the refractory period.

Somewhat similar results were obtained by making one injection and following that with another. If 0.0002 mgm. is injected, a vasoconstriction occurs and the uterine vessels are then refractory for 40 seconds to another injection of 0.0002 to 0.0006 mgm. of adrenalin. However, they are not refractory to 0.005 mgm. or more. After relatively large injections the refractory period is lengthened, for further injection of from 0.0002 to 0.0006 mgm. does not induce a vasoconstriction 5 minutes after the injection of 0.01 mgm. However, slightly different results were obtained by following one relatively large injection by a second injection of the same amount. Although the injection of 0.01 mgm. was followed by a vasoconstriction that lasted over 4 minutes, and this by a vasodilatation lasting 22 minutes, a second injection of the same amount within 5 minutes induced a vasoconstriction which lasted only 2½ minutes. It appears that small injections are followed by a refractory period and the larger by a period of fatigue or at least a period of lessened response. That the refractory period after the injection of small amounts is not merely a period of fatigue is shown by the fact that a second injection at the end of the refractory period induces as long a period of vasoconstriction as the first injection did, while the injection of a large amount of adrenalin even 10 minutes after a previous injection induces a shorter period of vasoconstriction.

There are two possible explanations of the vasoconstrictions that follow the injection of the smaller amounts of adrenalin. They were induced either by the adrenalin injected or frightening the animal at the time of injection. Four facts indicate that the first was the case. The injection of saline did not induce a vasoconstriction; injection while one transplant was in a refractory period and another in the resting period induced a vasocon-

striction only in the latter; the length of the vasoconstriction was proportional to the amount of adrenalin injected; and they were temporarily related, not to the insertion of the needle into the vein, but to the actual injection of the adrenalin.

The latent period is shortened as the amount of adrenalin injected is increased. When 0.01 mgm. is injected the latent time is equal to that found by Koppanyi (1930) in the iridial muscles, that is, 3 seconds. However, when from 0.0002 to 0.001 mgm. are injected the latent time is 14 seconds (allowing of course 3 seconds for the adrenalin to reach the eye).

Transplants that ruptured the eyeball. A single large transplant of a portion of the uterus was made to the eye of a 31 day old rabbit. The uterine horn at that age is so thin that some muscle was unavoidably included in the transplant which was so large that it nearly filled the anterior chamber of the eye. When the animal became sexually mature there was a marked bulging of the eye because of the growth of the transplant at this time. The animal was mated with a vasectomized male. About 38 hours later the eye ruptured and a portion of the transplant about 1.7 cm. long, and 0.8 cm. wide, 0.6 cm. thick, protruded through the lesion. Since this mass filled the opening there was very little escape of aqueous and this ceased after 3 hours. Since the extruded mass showed no signs of becoming infected and the animal did not seem to be in pain, it was decided to watch the transplant closely. Eight days later it began to decrease in size and continued to do so until 19 days after mating. This suggests that the growth and regression of the transplant was under the control of the secretion of the corpus luteum.

Since the extraocular portion of the transplant offered unusual possibilities for observation, the vascular cycle and the contractions of the uterine muscle as judged by movement of the mass were observed. After the animal was killed, the transplant was sectioned serially, stained, and examined microscopically for any changes in structure that might have been caused by changes in temperature, dehydration or trauma.

Vascular rhythm. The rhythmic vascular changes in the transplant were interrupted in vasodilatation, 7 hours after the animal had been mated and did not reappear until 10 days after mating. They had returned to their normal rhythm 13 days after mating and remained normal through the rest of the period of observation. A spontaneous cycle 65 seconds long persisted and in addition the typical vasoconstrictions of fright were observed.

Muscular contractions. When the mass protruding from the eyeball was relaxed, it hung down so that the lower portion of it was in contact with the cornea for about 1 cm. below the rupture point. When it contracted it seemed to shorten and become broader and the dependent portion seemed to be lifted forward so that it was separated from the cornea by a space from 0.1 to 0.2 cm. wide. The contractions observed were of two kinds, the rhythmic and the fright contractions.

Rhythmic contractions. As might be anticipated from Reynolds' (1930) work on uterine fistulae, no muscular contractions were observed in the uterine transplant for the first 12 days after mating. From that time on movements in the muscle mass were observed about every 110 seconds. It seems worthy of note that the muscular cycle was nearly twice as long as the vascular one and that it was more irregular temporally. Since the two cycles were of unequal length, it was impossible for them to occur simultaneously except at intervals.

Fright contractions. When the animal was frightened, the muscular contraction sometimes occurred 17 seconds later. The length of the latent period and the uncertainty of the response suggested that there might be periods in the muscular cycle similar to those in the vascular one. A contraction could be induced only during the resting phase and frightening the animal in any other phase had no effect on the muscular cycle. The contraction period lasted about 10 seconds, the refractory period about 30 seconds, and the resting period about 50 seconds, and the latent period 17 seconds.

Since the muscular and vascular cycles were of different lengths, frightening the animal had the four following results. Fright induced during the refractory period of both cycles induced neither vasoconstrictions nor muscle contraction. Fright during the refractory period of the vascular cycle and the resting period of the muscular cycle was followed only by contraction of the muscle. Fright during the resting period of the vascular cycle was followed by vasoconstriction, and fright during the resting period of both cycles was followed by simultaneous vasoconstriction and muscle contraction. The length of the contraction of the muscle was also roughly proportional to the degree of fright induced, lasting about 8 seconds after the animal was merely startled and as long as 25 seconds when the animal was severely frightened. Unfortunately it was impossible to ascertain by this method whether or not the intensity of the contraction was increased by frightening the animal more severely. After either the constrictions or the muscular contractions had begun, prolonging the period of fright did not prolong the response.

Injections of adrenalin (0.0002 to 0.0006 mgm.) cause a muscular contraction if the adrenalin reaches the eye during the resting period of the muscular cycle. If larger amounts are injected (from 0.005 to 0.01 mgm.) muscular contraction is induced in any period of the muscular cycle.

Reynolds states that the presence of the hypograstric nerve may account for the rhythmic muscular changes that he has recorded. In view of the fact that the rhythmic muscular contractions in the transplants have the same rhythm as those that he has recorded, the rôle of the hypogastric nerve seems to be excluded. Further, no nerves were found growing into the transplant, a fact which might have been anticipated since Raul (1930)

found no nerves growing into or out of transplants of fetal nervous tissue in the anterior chamber of the eye. Rhythmic contractility seems to be an inherent characteristic of uterine musculature and the vascular and muscular cycle is similar in several respects.

Discussion. The presence of a gradient in the rhythmic vascular changes in the uterus of the rabbit brings up the question as to whether or not the blood supply is the significant factor and also suggests that there may be loosely arranged myoneural elements in the uterus of the rabbit similar to the ones described in the human uterus by Hofbauer.

The lengthening of the resting period during the time of day when the vascular cycles are longest seems to indicate that the variations in the cycle found both in this species and in the guinea.pig during different parts of the day may be explained on that basis.

Since the vasoconstrictions that follow frightening the animal are not present after the adrenals have been removed it seems that these vasoconstrictions may be caused by the elaboration of adrenalin when an animal is frightened. Since contractions of the uterine muscle follow fright, the question arises as to what effect fright might have on the uterus during pregnancy and especially near the end of gestation, and what relation vasoconstrictions and uterine muscular contractions may have to the subjective experiences that woman undergoes when frightened.

Since rhythmic contractions of the uterine muscle occurred in transplants without any nerve supply, it would seem that these are an inherent characteristic of uterine muscle. Since they were not present during the period of pseudo-pregnancy, they may be arrested by one of the hormones that is present in increased amounts at that time.

This investigation indicated that from 0.0002 to 0.001 mgm. of adrenalin may be liberated per kilogram of body weight in the rabbit during 15 seconds of fright, but this method does not seem suitable to determine how much may be liberated during longer periods of fright. Cannon presented evidence that indicates that adrenalin is not continuously secreted by the resting animal. The results obtained in the present study may therefore represent the total amount of adrenalin that is secreted during fright rather than merely the increased amount that is secreted when the rabbit is frightened.

Cori (1931) has presented evidence that 0.0005 mgm. per minute of adrenalin will induce hyperglycemia in the rabbit. The results of the present study hence suggest that when a rabbit is merely startled, 16 times as much adrenalin is secreted per minute as is necessary to induce hyperglycemia and that in severe fright 48 times the amount of adrenalin required to induce hyperglycemia is liberated per minute. It also appears that from 3 to 12 times the amount required to produce glycosuria is liberated (Trendelenberg, 1923).

It is not definitely known that vasoconstriction or muscular constriction for longer than 25 seconds would be injurious to the uterus. However, the presence of a mechanism that prevents their continuation beyond 25 seconds suggests that they might be and that the utility of this mechanism and of the refractory period may be the prevention of injury to the uterus when fright is very prolonged.

SUMMARY

The uterine vascular cycle may, for the sake of clarity, be divided into 4 periods; the period of vasoconstriction, refractory period, resting period, and latent period.

The uterine muscular cycle may be divided into similar periods.

In a transplant of uterus in the anterior chamber of the eye of a rabbit, the two cycles may be independent.

Frightening an animal causes a contraction or a vasoconstriction only during the resting period.

The injections of from 0.0002 to 0.001 mgm. of adrenalin induced vascular changes in the uterine transplants similar to those that occur 17 seconds after a rabbit is frightened.

The technique of intraocular implantation appears to have been originated by J. Cohnheim (1877) and was used by Salomonsen (1879), Baumgarten (1880), and Klebs (1883). However, I believe that Schochet was not aware of this and I hence desire to acknowledge my indebtedness to him for the technique of implantation.

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CARDIOVASCULAR RESPONSE OF HEALTHY YOUNG MEN TO POSTURAL VARIATIONS AT VARIED TEMPERATURES[1]

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In this study we report the circulatory reactions of young men subjected to rather drastic conditions. Prolonged standing without even slight motion and without increase in breathing frequently results in fainting. The position imposes a definite burden upon the circulatory apparatus. Turner (1927a, 1927b, 1929) carried out a series of experiments on the circulatory response of college girls during prolonged quiet standing. She measured systolic, diastolic, and pulse pressures and the pulse rate. On the basis of these records she developed a system of grading circulatory efficiency which will be referred to as the "standing test." It was suggested that if standing could be divested of all muscular effort, the effects of gravity on the circulation might become even more definite and, further, if during this "passive standing" the subjects were subjected to high temperatures, their power to compensate might become still less. These expectations have not been realized, and our hope that we might be able to gather data helpful in determining normal circulatory fitness and ability to withstand high temperatures has not been realized.

METHODS. We have employed a tilting table which makes it possible to maintain the subject entirely passive while being moved from horizontal to vertical. The apparatus consisted of a padded table balanced on a horizontal axle. By means of an electric motor the table could be slowly revolved from the horizontal to the vertical position. This 90° excursion required about 18 minutes. A pointer indicated the varying angle on the dial bolted to the frame. An adjustable foot board was fitted to the subject's length, so that when his feet were resting flatly upon it, his shoulders were pressed snugly into the padded shoulder braces. The subject's knees were slightly flexed over a pillow placed on the table beneath them. Two wide, web straps were secured across the legs, one just below and the other just above the knees. A third strap was passed across the body at the level of the symphysis pubis. These arrangements allowed a maximum support to the body without compressing the abdomen. The heart rate and the systolic and diastolic pressures were recorded at every 10°, beginning at 0°. During the experiment the subject remained absolutely quiet and as relaxed as possible. The experiments were carried out in a room

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which was automatically maintained at any desired temperature. The absolute humidity at the four different temperatures used remained constant, since no moisture was introduced during the process of heating. A table somewhat similar, but without a motor to provide constant motion, was used by Turner, Newton, and Haynes (1930) as part of a series of experiments, the primary purpose of which was to demonstrate a slowly lessening return of blood to the right side of the heart as the subject slowly became upright.

Experimental observations. The subjects were medical students, ranging between the ages of 20 and 27 years. Just before each experiment, each man was given the Turner "standing test" in order to correlate and compare the tilting table response with that in the standing test. After the "standing test" the subject reclined for from 10 to 15 minutes until his blood pressures and pulse rate were again constant. He was then secured comfortably to the table in the horizontal position, and again pulse and blood pressure readings were taken until they became steady. These were the "0°" readings. The motor was then started, and the subject was revolved through 90° to a standing position. Without stopping the motor, the pulse rate and blood pressure readings were taken at every 10°, from 0° to 90°. After the subject reached the vertical, he was promptly returned to the horizontal level by hand, and a second 0° reading was taken. Three other readings were taken at 0° at intervals of 2, 4, and 6 minutes, respectively, after the return. Eleven experiments were carried out at room temperature, 19 at approximately 90°F., 24 at approximately 120°F., and 21 at approximately 130°F. The total number of subjects was 21; no subject was used more than four times at any one temperature. Eight of the subjects, for whom there were readings at each of the four temperatures, were placed in a group for special study, the idea being that if the temperature groups were composed of the same subjects at each temperature, the effect of the different temperatures would be brought out better than by a general group, in which some of the subjects would appear at one temperature and some at another. The results from this special group, however, were so similar to those of the general group that they have been omitted as unnecessary.

The data are presented in graphic and tabular form. Figure 1 consists of four graphs made from the composite responses of all subjects. The graphs for all four temperatures show certain general characteristics.

1. The systolic blood pressure remains nearly level throughout, with perhaps a slight rise up to 50° or 60° and a slight fall thereafter.

2. The diastolic blood pressure approaches the systolic curve up to 80°, and then declines from 1 to 2 mm. or remains level until 90°.

3. The resulting pulse pressure shows a steady diminution as 90° is approached.

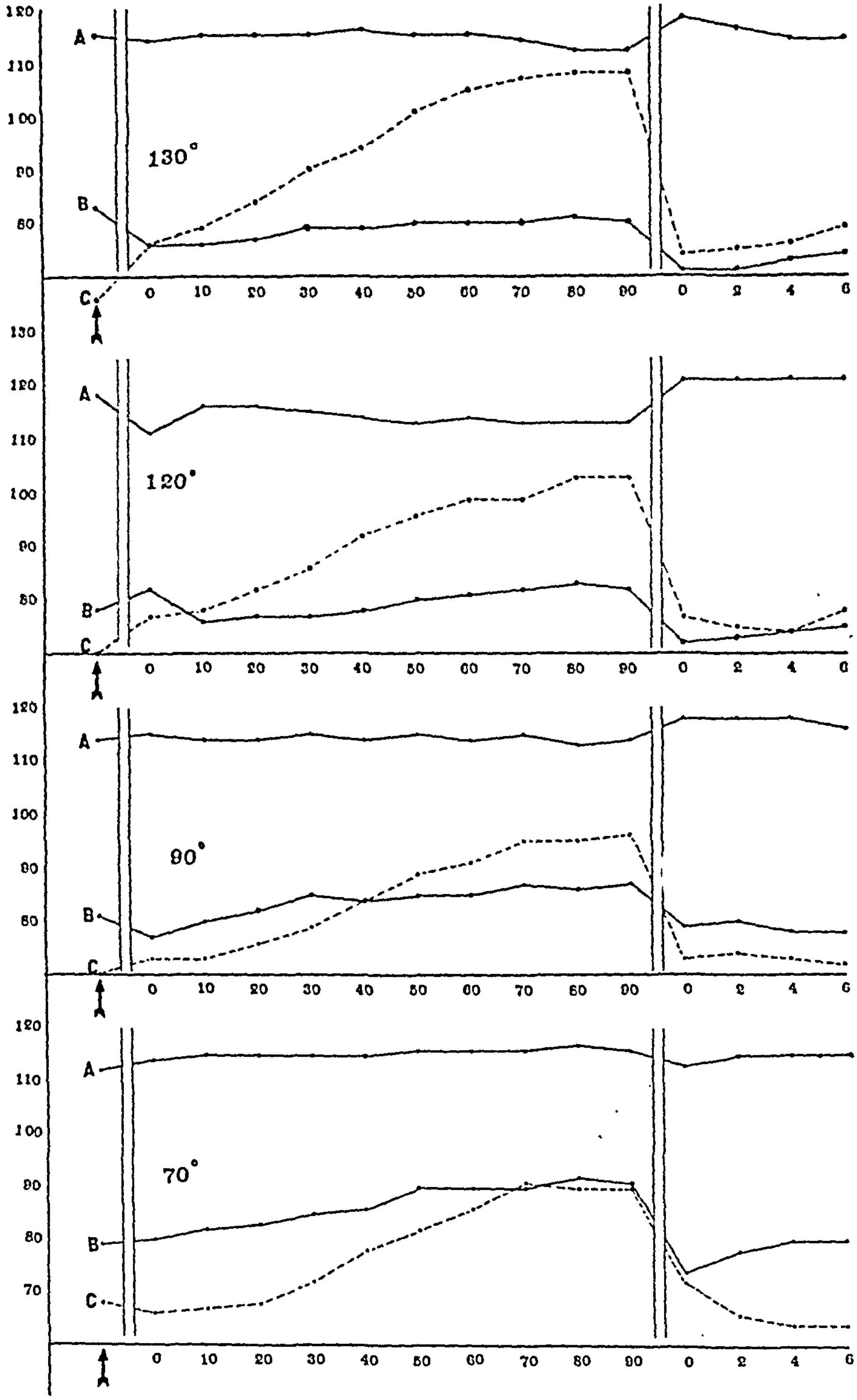

Fig. 1. Composite graphs showing pulse and pressure responses to posture at 70°, 90°, 120°, and 130°F. *Abscissae*, degrees of tilting. The arrow indicates "control" or reclining at room temperature; 0 to 90 are the angles of tilting from horizontal to vertical; 0 to 6 indicate the return to horizontal and 2, 4 and 6 minutes thereafter. *Ordinates*, both millimeters of mercury of pressure and beats per minute of pulse. *A*, systolic pressure; *B*, diastolic pressure; *C*, pulse rate. *Double lines*, breaks in continuity in the three curves *a*, as the subject moved from reclining outside to reclining inside the constant temperature room, and *b*, as the subject returned to horizontal from vertical.

4. The pulse response, far more sensitive than the pressures to changing posture, mounts steadily to a maximum at 80° or 90°.

5. When the subject is returned to the horizontal, the constant effect is a prompt widening of the pulse pressure in excess of that at the beginning of the experiment. This widening is produced by both a rise in the systolic pressure and a fall in the diastolic pressure. The pulse drops promptly to practically its initial reading. Pulse and pressure have not quite returned to initial readings at the end of 6 minutes, a time arbitrarily chosen to show the trend of the return to initial readings.

It was surprising to find such a slight change in response at the higher temperatures. Aside from a preliminary adjustment of pulse rate and pulse pressure between reclining at room temperature (control) and reclining in the heat (0°), the curves described by these readings at high temperature are very similar to those at room temperature. In short, the cardiovascular system is easily able to adjust itself to these higher temperatures. The subjects, aside from the discomfort in the heat, seemed on the whole to show little, if any, more tendency to faintness at the high temperatures than they did at room temperature. The procedure was stopped three times at 120°F. and once at 130°F. due to faintness of the subject. Two of these faints, one at 120° and one at 130°, occurred in the same subject, and all four occurred above the angle of 50°. All subjects perspired profusely, but none was markedly flushed.

The four graphs (fig. 1) show certain effects of the increasing temperature on the general response.

1. The systolic curve at room temperature rises slightly toward 90°. At 90°F. the curve is almost flat throughout, while at 120°F. and 130°F. it falls slightly toward the angle 90°. Whereas the systolic curve at room temperature is highest between 80° and 90° and falls as the subject is returned to the horizontal, at the elevated temperatures it is lowest between 80° and 90° and rises with the return.

2. The diastolic curve is at a lower level throughout at the higher temperatures, and the total rise is lessened.

3. The effect is to widen the pulse pressure as the temperature is steadily increased.

4. The pulse rate increased directly as the temperature.

In addition to the composite graph, a graph was prepared for subject S. G. P. (fig. 2), plotting the average of two determinations at each temperature. Although the curves are similar to those of the composite graphs, they show more extremes and more irregularity, as one would expect of an individual. These responses place him in the "Poor" group (see table 2).

Table 1a, compiled from each individual record, gives the number of significant drops in the pulse pressure at the angular intervals from 0° to

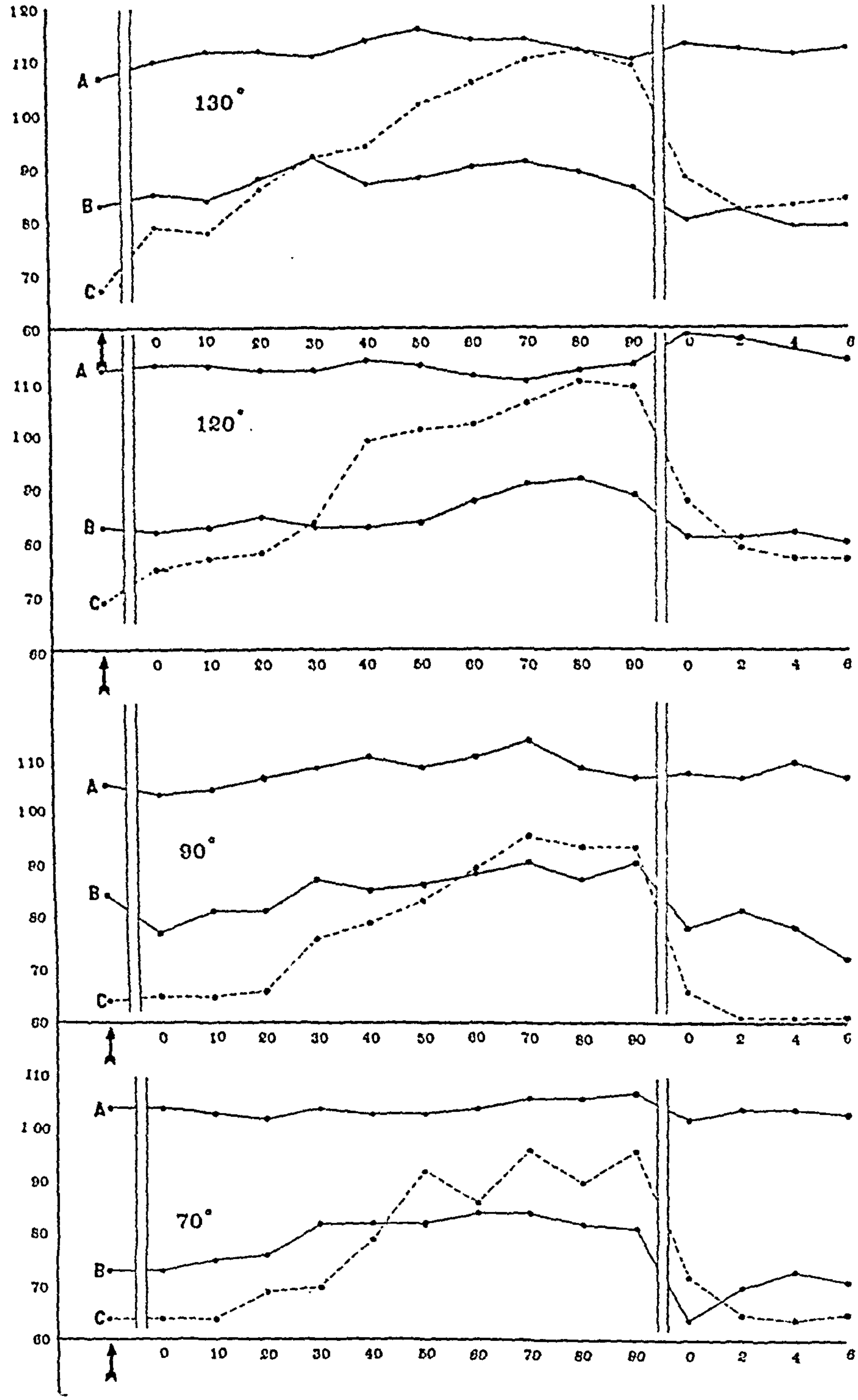

Fig. 2. Individual graph of subject S. G. P., showing pulse and pressure responses to posture at the four temperatures: 70°, 90°, 120°, 130°F. *Abscissae, ordinates* and *double lines* same as in figure 1. *A*, systolic pressure; *B*, diastolic pressure; *C*, pulse rate.

TABLE 1A

The number of significant drops in pulse pressure at the successive angular intervals

SIGNIFICANT DROPS IN PULSE PRESSURE DUE TO:	C-0	0-10	10-20	20-30	30-40	40-50	50-60	60-70	70-80	80-90	90-0	0-2	2-4	4-6
At 70°F.														
1. Fall in systolic pressure				1				1		1	1		1	
2. Rise in diastolic pressure		3	2	1		6	3	1	1			2	3	
3. Combination of 1 and 2					1									
Total number of significant drops		3	2	2	1	6	3	2	1	1	1	2	4	
At 90°F.														
1. Fall in systolic pressure	1		1		1	3	2	1	6					
2. Rise in diastolic pressure		7	4	4	3	1	3	1	2	2		2	2	
3. Combination of 1 and 2			1				1			1		2		1
Total number of significant drops	1	7	6	4	4	4	6	2	8	3		4	2	1
At 120°F.														
1. Fall in systolic pressure	2	2	3	1	4	5	2	3	5	2		2	4	
2. Rise in diastolic pressure	4	2	3	3	4	5	3	3	3	2		2		2
3. Combination of 1 and 2					1	1	2						2	
Total number of significant drops	6	4	6	4	9	11	7	6	8	4		4	6	2
At 130°F.														
1. Fall in systolic pressure	2		1	2	1	4	4		2	1		3	4	
2. Rise in diastolic pressure	1	1	3	5	5		2	4	6	1		3	2	3
3. Combination of 1 and 2		1		1	1			1		1				1
Total number of significant drops	3	2	4	8	7	4	6	5	8	3		6	6	4

TABLE 1B

Angle of occurrence of lowest pulse pressures in each experiment

TEMPERATURE	ANGLES									
	0°	10°	20°	30°	40°	50°	60°	70°	80°	90°
°F.										
70				1		3	3	1	3	3
90		1		3	2		3	1	9	3
120		1	1	1	2	2	1	4	7	7
130	1	2	1	1		2	3	1	9	6

90° at the four temperatures. A drop of 4 mm. or more between readings was arbitrarily selected as significant. The following observations are apparent:

1. In an examination of the tables for each temperature, one is unable to locate any definite areas of predominant pulse pressure drop, but (referring again to fig. 1) one sees that the summation of the pulse pressure drops acts to bring the maximum narrowing of pulse pressure between 80° and 90°.

2. The number of significant drops in pulse pressure due to systolic pressure fall is less than the number due to diastolic pressure rise. This is also seen in the composite graph (fig. 1).

Table 1b records, at each successive angle, the number of individual experiments which had their lowest pulse pressure at that point. In the experiments with the same lowest pulse pressure at more than one angle, each lowest reading was recorded. The greatest number of lowest pulse pressures fell at the angles of 80° and 90°, as is indicated in figure 1. However, what is not shown in the graphs is that a number of individuals gave their lowest pulse pressure reading at much lower angles—30° to 40° and up.

It is well known that at the angles approaching the vertical, various mechanisms come into play to assist the return of venous blood to the right heart. Notable among these are the aspirating effect of respiration, maintenance of adequate tone in the splanchnic bed, and the compression of peripheral veins by the muscles, as a subject shifts position or tenses himself. Variations of these mechanisms are seen in persons, faint from prolonged standing, who yawn, inspire deeply, and shift their weight constantly. Although each subject was instructed to keep absolutely quiet and relaxed during the test, the question arose whether a subject in such postural experiment was not helping his venous return, unconsciously, by increased respiration, as he was being raised toward standing. To investigate this point, tests were run upon 17 subjects at room temperature and in the usual manner, but with the subject breathing through a McIlhenny and Helm gas meter. In this way the minute volume of breathing was measured during each successive 10° interval. In figure 3 are plotted the pulse and pressures in the usual form. Curve D represents the litres per minute of air being breathed at each angle by the subject. (These readings were obtained by dividing the litres of air respired in each 10° interval by the time elapsed during the interval.)

The respiration curve is practically level up to 40°, but from 40° to 90° there is a steady rise, showing the compensatory increase in ventilation which was suspected. Although the subject was returned immediately to the horizontal from 90°, the curve shows that the air respired continued to increase nearly 1 litre per minute up to 2 minutes after the return and there-

after slowly decreased. This phenomenon is perhaps a mild and temporary orthopnea, produced by the momentarily increased volume flow through the pulmonary circuit and the resultant pulmonary congestion, when the blood from the dependent parts suddenly rushed back to the right heart—a mechanism similar to that described by Field and Bock (1925) in their investigation of the orthopnea in patients with cardiac decompensation.

Since the subject was fastened to the table and used a minimum of energy in maintaining his posture even at the vertical, we considered any

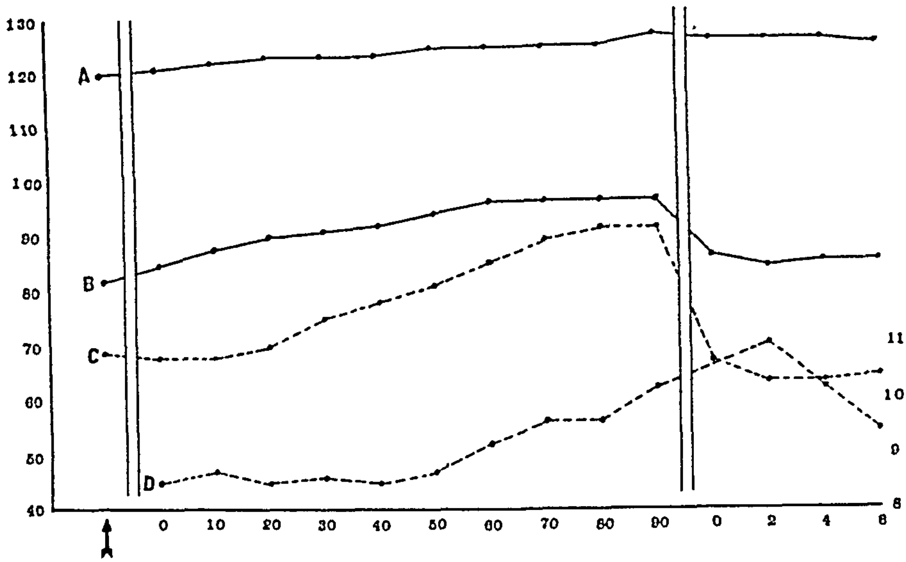

Fig. 3. Composite graph showing variation in respiratory volume accompanying postural change. *Abscissae, left ordinates* and *double lines* same as in figures 1 and 2. *Right-hand ordinates* indicate liters per minute. *A*, systolic pressure; *B*, diastolic pressure; *C*, pulse rate; *D*, liters per minute respired.

increase of ventilation due to increased metabolism at the upright positions to be negligible.

On the basis of the 92 tests run during this investigation and on the basis of 60 similar preliminary tests, run at a previous time by one of the authors, a few general standards have been evolved for judging what we consider good or poor responses. The results are too irregular, in our estimation, to warrant a detailed scoring system for cardiovascular efficiency, but in our judgment responses may be grouped in the following general manner:

	Good	*Poor*
1. Systolic:	a. Remaining nearly level	a. A decided fall
	b. No sharp rises or falls between readings	b. Marked variations between readings

	Good	*Poor*
2. Diastolic:	a. A gradual rise but not enough to lower the pulse pressure below 18 mm.	a. A rise lowering the pulse pressure below 18 mm.
	b. No sharp variations between readings	b. Sharp variations between readings
3. Pulse Pressure:	a. Never below 18 mm. and without any sharp variations between readings	a. Falling below 18 mm. or showing sharp fluctuations between readings
4. Pulse Rate:	a. At start not over 85 or under 45	a. At start outside these limits
	b. A moderate rise throughout the test. (In high temperatures this rise may be larger)	b. An extreme rise throughout the test
	c. No sharp variations between readings	c. Sharp variations between readings

It will be noted in this tabulation that the absence of sharp variations in any of the four readings is given a prominent position in the estimate of a favorable response. Indeed, in the absence of any extreme total variation, we regard this as the most important single item. The pressure readings, also, are regarded as of at least as much significance as the pulse rate in the cardiovascular response. Since much consideration has never been given to the return of pressure and pulse readings to constant, these data have not been included in the table.

On the basis of this table the tests were separated into a "Good" group of 19, a "Poor" group of 15, and an intermediate "Fair" group of 30. This was done to compare the estimate of good, fair, and poor with the actual "Standing" scores of the Turner scoring system, which were also taken on each subject. Table 2 shows that the average Turner score for the "Poor" group, 4.7, is definitely lower than the averages of the other two. Unfortunately, the average Turner score for the "Good" group, 7.4, is just below that for the "Fair" group, 7.9, whereas we had hoped to find it significantly larger. Both the tilting test and the standing test frequently show responses for the same subject which differ so much on different days that it is hard to conceive that the efficiency of his cardiovascular mechanism has varied to an equal degree. Both tests frequently indicate disparity of cardiovascular efficiency among a given group of subjects quite contradictory to their relative physiques. The normal limits are wide, and the tests fail to bring out any constant, clear-cut variations in response such as would justify precise grading It would seem, therefore, that these two methods of observing the cardiovascular system are but rough estimates, at best, of the true condition of the circulation, and that any attempt at a scoring more detailed than an estimation of good, fair, and poor is

artificial. It is, however, safe to say that the members of the "Good" and "Fair" groups tended to be better physical specimens and more athletic than the members of the "Poor" group.

TABLE 2

Comparison of the tilting table groups and the corresponding Turner score

GROUP	NUMBER IN GROUP	AVERAGE TURNER SCORE	DIVISION OF TURNER SCORE	NUMBER OF TILTING TABLE GROUP IN DIVISION	PER CENT OF TILTING TABLE GROUP IN DIVISION
Good	19	7.4	13-up	3	16
			10-12	4	21
			7-9	4	21
			4-6	3	16
			1-3	3	16
			0-below	2	10
Fair	30	7.9	13-up	2	7
			10-12	6	20
			7-9	13	43
			4-6	6	20
			1-3	3	10
			0-below	0	0
Poor	15	4.7	13-up	0	0
			10-12	0	0
			7-9	6	40
			4-6	6	40
			1-3	1	7
			0-below	2	13

SUMMARY

1. Tilting subjects from a horizontal to a vertical position—head up—has certain effects upon the cardiovascular response at all temperatures:

a. The systolic pressure curve remains nearly level, while the diastolic pressure steadily approaches it as the vertical position is neared. The resulting pulse pressure shows a physiological narrowing.

b. The pulse rate rises steadily to a maximum at 80° or 90°.

c. When the subjects are returned to the horizontal, the pulse pressure immediately widens in excess of the reading at the commencement of the experiment. The pulse rate rapidly drops.

2. The effects of high temperature are as follows:

a. The systolic pressure tends to fall slightly at the higher temperatures, but since the diastolic pressure curve is lowered more than is the systolic pressure curve, the pulse pressure is wider than at room temperature.

b. The pulse rate increases directly as the temperature.

c. There were three instances of fainting at 120°F. and one instance at 130°F., all occurring above the angles of 50°.

3. Tilting experiments were carried out in which the subject's ventilation was measured by means of a special spirometer. The results show a steady increase in ventilation as the subject is tilted from horizontal to vertical, a mechanism which by its aspirating effect helps to return the blood to the right heart in erect postures.

4. As regards a scoring system of cardiovascular efficiency, the writers feel that the data obtained in these experiments justify only a general estimate of any given response, such as "Good," "Fair" or "Poor."

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THE HYDROGEN ION CONCENTRATION OF THE GASTRIC JUICE OF FETAL AND NEWBORN WHITE RATS

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In the course of an investigation on the erythrocyte and hemoglobin content of fetal and newborn white rats, it was frequently noted that the stomachs of these animals were filled with a clear fluid. This fluid might represent the secreted products of the gastric glands or it might be amniotic fluid that had been swallowed. The simplest test that occurred to us to reduce these possibilities to one, was that of testing the gastric contents and the amniotic fluid for their reaction. Accordingly, the reaction was obtained first with litmus and later with standard buffered solutions of differing pH values. It was found, for example, that the gastric content of a fetus on the 18th day of gestation was distinctly acid to litmus and had a pH of 6.4 while the amniotic fluid was alkaline to litmus and had a pH of 7.4. It seemed fairly safe to conclude, therefore, that the gastric content was made up for the most part, at least, of gastric juice.

The query arose as to when the gastric juice began showing an acid reaction and how rapidly the acidity increased to that value usually found in adult animals. Data for answering the above questions were obtained in the following manner.

METHOD. Adult male and female rats of known fertility were placed on our stock diet.[1] By the use of the vaginal smear technic the period of heat was determined and the males placed with the females. The presence of sperm in a subsequent smear was considered as indicative of a positive mating. This was further confirmed if the female showed a placental leak on the fourteenth day following. The age of the fetuses was determined by this sign. As early during pregnancy as proved practicable, the female would be anesthetized and the fetuses removed one at a time by the abdominal route. The fetus' abdomen was opened and the stomach grasped by a fine curved forceps in such a manner as to include the esophagus and pylorus. As soon as the stomach was removed and while still in the grasp of the forceps, it was immersed in distilled water, rinsed and partially dried. The stomach was then held over the hollow of a micro-

[1] Cracked yellow corn, 35; cracked wheat, 35; alfalfa meal, 4; linseed oil meal, 16; casein, 5; calcium carbonate, 0.5; sodium chloride, 0.5 and dried yeast, 4.

culture slide[2] and carefully slit open. A free edge was grasped in the forceps and, by means of a fine pipette, doubly distilled water was slowly dropped over the gastric mucosa in order to wash off the gastric juice and add to the latter until there was sufficient fluid barely to fill the depression. One or two drops of an indicator were added and a cover slip sealed in place by a ring of vaseline.

A complete series of buffered solutions ranging in pH from 1 to 10 was set up.[3] A series of culture slides was placed in a row and the depressions nearly filled with those buffered solutions whose pH values would cover the range of gastric contents. The indicators used were as follows: thymol blue with a pH range of 1.2 to 3.0; methyl orange 3.0 to 4.5; methyl red 4.5 to 5.9; brom thymol blue 6.0 to 7.4 and phenol red 7.4 to 8.0. It was always necessary to sacrifice a few fetuses in order to determine the approximate range of pH and the indicator to be used. When the slides were all set up a few moments were allowed to elapse for the complete and uniform development of color. It was then only a question of matching the unknown with a known. This was a very easy procedure and one giving a high degree of accuracy as determined with small quantities of solutions of known pH. Determinations with the potentiometer (Youden) gave satisfactory agreement.

It was found impractical to work with fetuses younger than 17 days. The stomachs were too small and contained practically no fluid. The tissues were so fragile that we could not feel assured that we were not mixing tissue juice with gastric contents. For example, the stomach of a fetus removed on the 15th day gave a pH of 7.2. With the newborn animals, care had to be taken to get them before they had suckled. With those animals of greater age (from 1 to 21 days) a slightly different precaution had to be observed. Food was always present in the stomach. These animals were separated from the mother and kept in a warm place for from 2 to 4 hours before they were killed and the stomachs examined. In this way the food was considered as a test meal and the examination conducted at approximately the height of acid accumulation. In a few animals the preceding assumption was tested by injecting, previous to the examination, a solution of histamine. The solution was made up so that animals receiving 1 drop per 5 grams of body weight received 0.1 mgm. of histamine per kilogram of body weight. In the 13 trials of this nature, no values were obtained leading us to suspect that the routine procedure was giving acid values that were too low. In chart 1 the points surrounded by a circle represent the determinations obtained after the injections of histamine.

[2] Micro-culture slide 75 × 25 mm. with a cylindrical depression 3 mm. deep by 15 mm. diameter. Stock number 38230, Braun-Knecht-Heiman Co., San Francisco.

[3] We wish to express our thanks to Dr. H. D. Haskins of the Biochemistry Department for the assistance he gave us in preparing these solutions.

The Boas test for HCl was used in a few instances in post-natal rats only but because of irregular results was not continued throughout the study. Table 1 gives the results obtained. Keene and Hewer (1) used the Gunsberg reagent in testing for HCl in the stomachs of human fetuses of different ages. They tested the glycerine extract of fresh stomachs as well as the fresh material itself. Their results appear in table 2. We feel that, in our experience at least, a positive result may be considered as definitely indicative of free HCl but that a negative result cannot be considered as

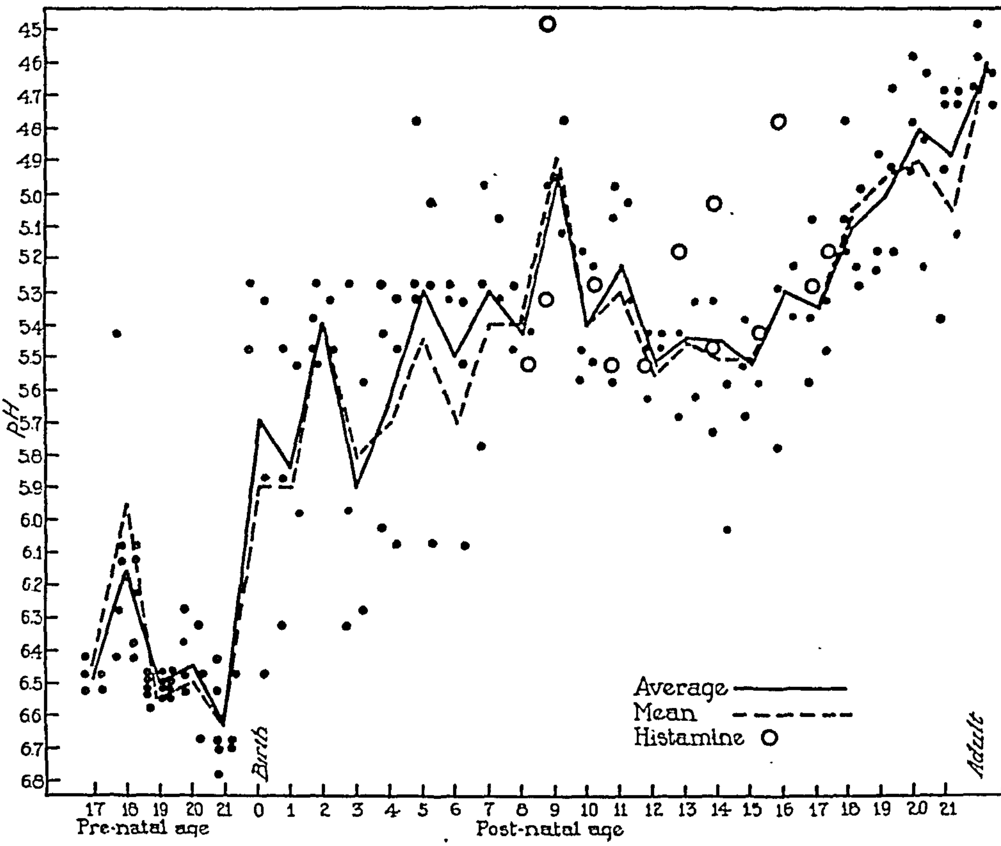

Chart 1. The pH value of the gastric juice of white rats varying in age from the 17th day of gestation to the 21st day of lactation.

conclusive evidence of the lack of HCl production. Keene and Hewer's results were not complicated by the presence of food in the stomach and still 5 out of 24 tests at full term were negative. Our negative results occurred only in the older animals where it was difficult to get them with empty stomachs.

Chart 1 summarizes the data obtained in respect to the pH of the gastric contents of fetal and newborn rats of different ages. It will be seen that during the last days of gestation there is a tendency for the gastric contents

to reach a peak in acidity and then rapidly recede so that at the end of the intra-uterine period the pH of the gastric juice was slightly greater than 6.6. Immediately after birth, however, there was a remarkable rise in acidity,—the pH changing from about 6.6 to 5.7. From this time on the changes in the pH of the gastric juice may be grouped into three phases: first, the first week of lactation where a gradual but definite increase in acidity occurs; second, the second week of lactation where a slight drop in acidity occurs, and third, the last week of lactation where there is another marked rise in acidity. At the end of lactation the pH of the gastric juice still is slightly greater than that obtained for adult animals (4.6).

TABLE 1

AGE	BOAS TEST
12 hours	2 positive
3 days	2 positive
4 days	1 positive
6 days	1 negative
18 days	2 negative

TABLE 2

AGE	GUNSBERG TEST	
	Fresh stomach	Glycerine extract
16 weeks		3 negative
17 weeks		1 negative
18 weeks	1 negative	1 negative
19 weeks	1 positive	1 negative
22 weeks	1 negative	2 negative
24 weeks	1 neg. 1 pos.	5 neg. 1 pos.
30 weeks		1 negative
32 weeks	1 positive	1 positive
Full term	8 pos. 1 neg.	11 pos. 4 neg.

Millet (2) upon determining the pH of various tissues of fetal rabbits found that "the fetal tissues become progressively less acid with increasing age of the fetus." For example, the pH of guinea pig's blood 1 hour after birth was 6.02; 3 hours later it was 6.4 and 7.5 hours later 7.16. Millet quotes Mlle. Mendeleef as observing that fetal blood of guinea pigs at half term was distinctly more acid than maternal blood. Millet himself showed the following relationship between fetal and maternal blood (table 3). Our results of 7.2 for the pH of the stomach of a fetus of 15 days and of 6.48 as the average for the 17th day indicate that prior to the 18th day for the rat, the acidity is not so marked. Furthermore, a pH of

7.4 for the amniotic fluid of an 18 day fetus shows no correspondence to the acidity of the gastric contents. It is probable, therefore, that in the rat up to the 18th day of gestation, the acidity within the gastric lumen is considerably influenced by the reaction of the tissues in general and that

TABLE 3

	DAY OF GESTATION					BIRTH
	20	23	24	26	29	
pH Heart	5.78	6.82	7.06		7.06	7.41
pH Liver	5.98	6.58	7.05	6.98	7.03	7.11
pH Kidney	5.55	6.68	6.78	6.35 6.67	6.43	7.11
pH Amniotic fluid	5.74					
pH Heart's blood			6.50	6.88		7.19*
pH Mother's blood			7.35	7.21		

* 12 hours later the pH was 7.35.

after this time the reaction is appreciably augmented by an intrinsic mechanism.

The method that we used in obtaining the pH of the gastric contents does not rule out the contributing influences that lactic acid or carbon dioxide may play. The point might well be made, therefore, that the acid pH values obtained during the period of gestation might well represent the influence of all three acids instead of HCl alone. In view of the work cited above this might be the preferable explanation. If it is true that fetal tissues during the middle of gestation are more acid than at the beginning or the termination of this period, it seems necessary to inquire into what various factors might contribute to this condition and to the decided changes which occur as gestation advances or as the result of the initiation of external respiration.

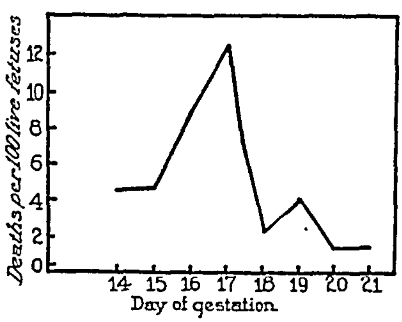

Chart 2. The death rate of fetal rats per 100 live fetuses per day of gestation.

First let us ascertain whether or not there occurs an intra-uterine crisis of serious enough nature to limit the size of the litter. A survey of our data shows that 82 pregnant females examined at varying stages of pregnancy had a total of 643 live young and 32 resorptions. Chart 2 graphically depicts the number of deaths per 100 live young by age of the fetus. The

average size of the litters was reduced from 8.6 to 7.3 during the last 8 days preceding birth. The greatest prenatal mortality was during the 17th day of gestation. Donaldson (3) shows that there is a marked decrease in growth increments of the fetus after the 17th day. It is during this period that the relatively rapid growth imposes the greatest load upon the oxygen carrying tissues. Any deficiency in oxygen transport would immediately be reflected by a stimulation of the hematopoietic tissues and the development of a condition of asphyxia the severity of which would depend upon the extent of the oxygen shortage. Products of anaerobic oxidation such as lactic acid would appear and tend to alter the reaction of the tissues. Those fetuses remaining alive through this critical period would represent either those individuals which were able to compensate sufficiently to meet the demands of the occasion or those sufficiently endowed with the essentials to maintain an existence barely within the limits compatible with life.

Toward the end of the period of gestation through the sacrifice of 15 per cent of the original litter and through the rapid development of erythrocyte and hemoglobin, the oxygen deficiency is relieved. After birth, with greatly increased oxygen tensions, the number of red cells and the amount of hemoglobin is actually reduced.

On the 9th day of lactation a gradual decrease in gastric acidity begins. The decrease reaches its lowest level on the 15th day. From this time on a gradual increase in acidity occurs. Whether or not this drop in acidity (4.94 to 5.52) represents a marked enough change to be significant is difficult to say. Mention should be made, however, of the fact that it is during this same period that the red cells and hemoglobin (especially the latter) reach the lowest level. As soon as the lactating rat begins to partake of the mother's diet there is a rapid increase in the hemoglobin and red cells. The existence of any possible relationship between the amount of acid secreted into the stomach and the oxygen carrying capacity of the blood must, for the time at least, remain an open question. It must be pointed out, however, that there is a frequent parallelism running between gastric acidity and the number of red cells and the amount of hemoglobin.

In the human fetus, Keene and Hewer (1) found that there was free HCl formed in the stomach as early as the 19th week (fresh stomachs) and the 24th week (glycerine extracts of fresh stomachs (table 3). McRobert (4) determined the pH of the gastro-intestinal tract of white rats ranging in age from 4.5 to 12 months. He found the pH of the stomach 5.5 at the cardia and 2.39 at the pylorus. Grayzel and Miller (5), working with dogs fed a normal diet, observed that the pH of the gastric contents averaged 3.68. In guinea pigs, Redman, Willimot and Wokes (6) obtained an average pH of 3.8 and in adult rats found a pH of 4.2. They carried on some investigations of the intestinal contents at various levels in young rats ranging in age from 4 to 12 weeks and found that "the small intestine

tends to be more alkaline than in adults." No determinations on the stomach were reported.

In some previous work done in this laboratory (unpublished data) on the red cell count and hemoglobin content of the blood of fetal rats as contrasted with that of the newborn and nursing rats, it was observed that there was a sharp change in level between the results obtained on the 21st day of gestation as compared wth the 1st day of lactation. It is obvious that there is an extremely marked change in living conditions in the two periods. That there should be such significant changes in some of the physiological balances existing within the body at such times is very interesting and may contribute to a better understanding of the causes which mark this time as a very crucial period in the life of the individual. Eastman (7) has shown that one of the factors contributing to make this a critical period in life is the different oxygen tensions to which the blood of the individual is exposed before and after birth. Physiology has established the fact that low oxygen tensions will cause a marked rise in the red cell count. This explains very well the marked differences in red cell counts of pre- and post-natal rats to which reference was made above. Whether or not it is a contributing factor to the detemination of the character of the gastric juice cannot be said at this time. There are those, however, who hold that there is a connection existing between the amount of carbon dioxide being eliminated from the body and the amount of HCl being produced by the gastric mucosa.

As already pointed out, the pre-natal limitation of litters occurs with the highest frequency of fetal deaths between the 14th and 18th day of gestation. It is rather common experience in the laboratory that the highest incidence of post-natal deaths occurs during the latter half of the second week and the first half of the third week of lactation. Whether or not the decrease in acidity at this time is in any way related to this occurrence remains for future work to decide.

SUMMARY

pH determinations on the gastric contents of 43 fetuses covering the last 5 days of gestation shows that during this period the acidity reaches a maximum on the 17th day and thereafter declines. Similar observations made on the gastric juice of 120 lactating rats covering the entire 21 days of the lactation period shows five noteworthy features: *a*, there is a marked increase in acidity of the gastric juice as soon as the animal is born; *b*, during the first week of lactation there is a gradual increase in acidity; *c*, the middle third of the lactating period shows a slight decrease in acidity; *d*, the latter third of lactation shows a sharper increase in acidity than the first week; *e*, the acidity at the end of lactation is not as great as is found in the adult animal. The association of the highest incidence in pre-natal and post-natal mortality with these changes is commented upon.

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A COMPARISON OF THE RELATION BETWEEN THE RATE OF UREA EXCRETION AND THE AMOUNT OF RENAL TISSUE IN THE DOG AND OTHER MAMMALS

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Taylor, Drury and Addis (1923) have demonstrated that in rabbits there exists a linear relationship between the magnitude of the ratio: $\frac{\text{Urine urea rate}}{\text{blood urea concentration}}$ and the weight of the kidneys. A similar relationship has been found (MacKay and Raulston, 1931) in the rat and the ratio value per unit of renal weight was practically the same as that for the rabbit. The indirect figures for man are suggestive (Taylor, Drury and Addis, 1923) and it seemed possible that this ratio: renal weight figure might hold for mammals generally. A perusal of the data available for the dog however suggests that the value of the ratio: $\frac{\text{urine urea rate}}{\text{blood urea concentration}}$ per unit of kidney weight may be definitely higher than in the other species. Most of the data for the dog (Marshall and Davis, 1914; Pepper and Austin, 1915; McEllroy and Pollock, 1921) only indirectly give us information upon the point in question here. Ralli, Brown and Pariente (1931)[1] have compared the rate of urea excretion in the dog with the kidney weight but unfortunately at low urine volumes under which conditions the urea excretion: urine volume relationship is for the dog an unknown quantity. In any case the range of distribution of their kidney weights is hardly large enough to give rise to any general conclusions.

In the present experiments the conditions set down by Addis for obtaining constancy in the urea ratio for a given subject were rigidly observed. These are essentially the provoking of an adequate diuresis by water

[1] These investigators have used the term "maximum blood urea clearance" suggested by McIntosh, Möller and Van Slyke for the urea ratio as originally used by Addis multiplied by 1.67. Naturally there is an obvious advantage in uniformity of nomenclature but we have hesitated to adopt this designation for the ratio $\frac{\text{urine urea rate}}{\text{blood urea conc.}}$, as used here and in the papers of McIntosh, Möller and Van Slyke, is not the "maximum" figure which may be obtained for this measurement. It may be increased by excessive diuresis, caffeine administration, protein ingestion and other factors.

ingestion and the administration of urea three hours before observations are commenced, during fasting 12 hours after the last ingestion of food. Three hundred milligrams of urea and 10 cc. of water per kilo of body weight were first given by stomach tube and then 5 cc. of water per kilo each hour thereafter until observations were suspended. Three hourly observations were made on each animal. The dog was then killed with cyanide and the kidneys removed. After decapsulation, removal of the pelvis fat and slicing followed by blotting with filter paper to subtract as much of the adherent blood and urine as possible, the organs were weighed. The figures obtained from ten normal dogs are summarized in table 1. The constancy of the urea ratio per gram of kidney makes it obvious that as in the rabbit and rat there is a direct relationship between the kidney weight and the rate of urea excretion in the dog. The values per gram

TABLE 1

DOG NO.	BODY WEIGHT	URINE VOLUME	URINE UREA	BLOOD UREA	RATIO: URINE UREA RATE / BLOOD UREA CONCENTRATION	KIDNEY WEIGHT	RATIO PER GRAM OF KIDNEY
	kgm.	*cc. per hr.*	*mgm. per hr.*	*mgm. per 100 cc.*		*gm.*	
1	2.1	65	524	84.1	6.24	14.2	0.44
2	3.0	40	345	41.8	8.25	23.6	0.35
3	6.0	160	385	37.9	10.14	35.0	0.29
4	11.4	105	778	45.8	17.00	45.7	0.38
5	16.0	110	1,015	63.4	16.00	47.2	0.34
6	18.4	215	1,310	71.0	18.42	46.1	0.40
7	19.0	98	1,140	46.7	24.44	59.7	0.41
8	22.3	205	1,185	50.5	23.50	72.7	0.32
9	24.5	180	1,426	41.4	34.50	93.3	0.33
10	30.5	270	1,486	34.6	43.00	113.2	0.38

of kidney show however that per unit weight the dog's kidney is more active than that of the rabbit or rat. The average figure for the rabbit was 0.176 and the rat 0.198 in comparison with 0.364 per gram found here for the dog. In man this relationship obviously cannot be determined directly but it is possible to compare the urea excretion ratio with the kidney weight as predicted from body surface by methods to be published elsewhere. For comparison with the dog the expected kidney weight has been compared with the Addis urea excretion ratio in a group of normal male subjects.

The data obtained here for man and the dog are compared in figure 1 with the data of Taylor, Drury and Addis (1923) for the rabbit and MacKay and Raulston (1931) for the rat. It is evident that the activity of the kidney per gram of tissue as measured by the urea ratio is practically

the same for all four species except the dog. Here the renal activity in relation to kidney weight is significantly higher. The reason for this is not evident. We are unaware of any great differences in the structure of

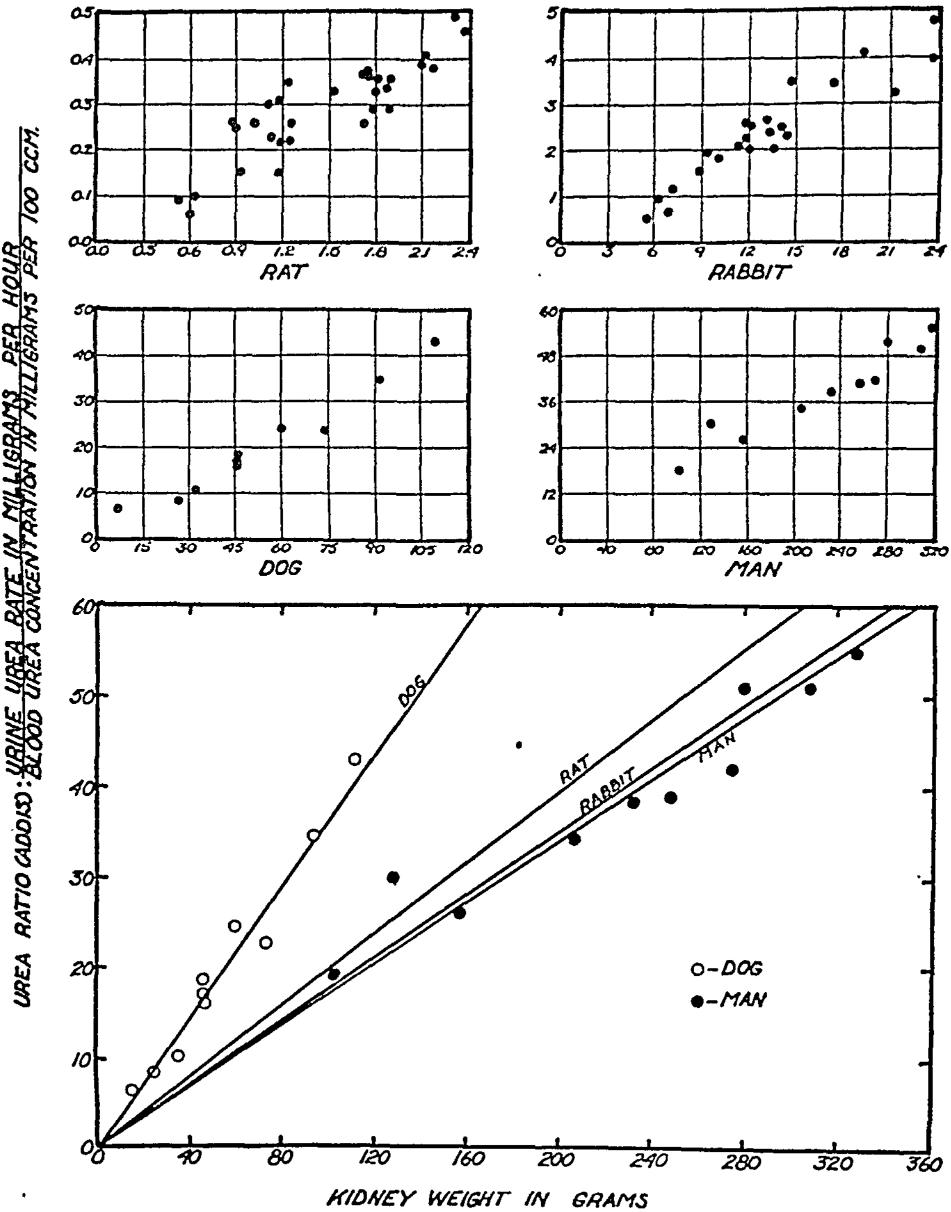

Fig. 1

the dog's kidney in comparison with this organ in the other species. We find on recalculating Vimtrup's data (1928) that the dog, man and the rat have 31,800, 31,200 and 32,200 glomeruli per gram of kidney respectively. Kunkel (1930) finds a diameter for the renal glomeruli in the dog

of 186 micra in comparison with 151 micra for the rat and 142 micra for the rabbit but records only 13,300 glomeruli per gram of dog kidney in contrast with 33,000 and 31,000 respectively for the rabbit and rat.

It occurred to us that the dog's renal tubules may absorb less of the glomerular filtered urea than appears to be taken up by those of the frog (MacKay and Oliver, 1930) or rabbit (MacKay and MacKay, 1930). If we consider the possibility that Rehberg's (1926) contention that creatinine reaches the urine solely by glomerular filtration and that none is reabsorbed in the tubules is correct, we may examine this question through a simultaneous comparison of urea and creatinine ratios. This was done in a number of dogs with results essentially the same as those since reported by Jolliffe and Smith (1931a). The creatinine ratio is definitely higher than the urea ratio in the dog as in the rabbit (MacKay and Cockrill, 1930) and man (MacKay, 1930). The only other possibility which suggests itself is that man, rabbit and rat are either omnivorous or herbivorous while at least in our material the dog is entirely carnivorous and it has been shown (Addis and Drury, 1923) that protein increases the $\frac{\text{urea ratio}}{\text{kidney weight}}$ relationship transiently when administered during the period of observation. The effect of the maintenance diet preceding the observation is unknown. Jolliffe and Smith (1931b) have found that for a given dog the urea ratio fluctuates with the amount of protein in the diet. This however aids us little for it is probable that the renal weight of the dog depends upon the protein intake just as in the rat (MacKay and MacKay, 1931). Observations upon this point and the effect of the maintenance diet preceding the measurement of the urea ratio upon the $\frac{\text{urea ratio}}{\text{kidney weight}}$ relationship are now in progress.

Since in all of the four species examined here there is a direct relation between the kidney weight and urea excretion ratio it follows that the same thing is true between the ratio and body surface for it has been shown by Stewart (1921) for the dog, Taylor, Drury and Addis (1923) for the rabbit and, MacKay and MacKay (1927) for the rat and elsewhere (MacKay, 1932) for man that the kidney weight bears a direct relation to body surface.

SUMMARY

The urea excretion ratio: $\frac{\text{urine urea rate}}{\text{blood urea concentration}}$ bears a direct relation to renal weight in the dog, rat, rabbit and man. In the dog the ratio value per gram of kidney weight is a great deal higher than for any of the other three species examined in all of which the relationship is essentially the same. The possible reasons for this are discussed.

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CAPILLARY COUNTS IN RESTING AND ACTIVE MUSCLES[1]

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In the course of a study in this laboratory of the activity metabolism of single mammalian muscles in situ (Martin, Field, Hall and Field, 1931) it seemed desirable to have precise information of the details of the capillary circulation in the muscles under various conditions, and especially in rest and during exercise. The important pioneer work of Krogh on the capillary circulation of muscles (1922) was done on muscles other than the gracilis (in the dog the muscle examined by Krogh was the semimembranosus) whereas the work in this laboratory has been done on the gracilis, and it is quite conceivable that findings from one muscle might not hold closely for another, even in the same animal. We accordingly undertook the series of observations reported in this paper. All the work was done on gracilis muscles of dogs, except in one experiment in which the semimembranosus muscles were used to enable us to compare the results obtained by our method with those obtained by Krogh, who used a somewhat different injection technique.

Methods. We made the capillaries demonstrable by flooding the intact muscle in situ with undiluted india ink under the arterial pressure prevailing at the instant of injection, the pressure being recorded throughout each experiment from a cannula in a carotid artery. The gracilis muscle of the dog lends itself admirably to this procedure, both on account of its own superficial position and because of the ready accessibility of its main nutrient vessels. A cannula directed centrally was introduced into the femoral artery just distal to the point of origin of the gracilis artery. A syringe of about 15 cc. capacity, filled with india ink, was connected to the cannula, air bubbles being carefully excluded in making the connection. At the desired moment a clip that had been previously placed on the syringe tube was removed and a considerable volume of ink injected into the femoral artery. The rate of injection was so adjusted as to insure that the blood entering the muscle was wholly or almost wholly replaced by ink. Both the muscle and the venous outflow blackened promptly, and as soon as the latter blackening was clearly evident, a matter of ten seconds or

[1] A preliminary report on part of this work was published in the Proceedings of the Society for Experimental Biology and Medicine, 1930, xxviii, 33.

thereabouts, the gracilis artery and vein were clamped off, as were also both ends of the muscle by means of large hemostats previously placed in position. The muscle, which had been freed from the adjoining tissues except at origin and insertion prior to the injection, was then quickly cut away, outside the hemostats, and with the aid of the latter plunged into a large beaker of Bouin's fluid for fixation. The muscle shortens and thickens somewhat when cut away from its attachments, and we endeavored to compensate for this to some extent by holding the hemostats apart and the muscle thus under some tension during the first few minutes of fixation. It is not likely that we duplicated the normal tension but we were successful in avoiding distortion of the muscle, our fixed preparations showing, practically without exception, straight fibers throughout.

After preliminary fixation of about an hour the muscle was cut into strips of suitable size for sectioning and the fixation continued for about twenty-four hours. The standard procedure for making paraffin sections was then followed. Transverse sections were cut in thicknesses of nine to fifteen microns, enough being made from the different strips to ensure that all parts of the muscle were sampled. In all the preparations from animals in which amytal was used as the anesthetic (the technical work on these was done by E. W.) the sections were stained on the slides with Delafield's hematoxylin and eosin. Twenty-two muscles were treated thus. In the preparations from etherized dogs, decerebrate dogs, and dogs into which amyl nitrite was injected (the technical work on these was done by M. M.), the sections were mounted without staining. We found, as a matter of fact, that by discontinuing the washing with alcohol before the last traces of yellow coloration from the Bouin's fluid had disappeared we obtained sections in which all the features significant for our purpose, i.e., the boundaries of the fasciculi, the outlines of individual fibers, and the injected capillaries, stood out more sharply than in the stained preparations. Eleven muscles were treated thus.

The method of demonstrating capillaries by india ink injection has been criticized by Hartman, Evans and Walker (1929) on the ground that ink particles have been observed by them to catch at the origins of open capillaries, preventing ink from entering them and thus excluding them from subsequent counts. We are, of course, unable to say to what extent this may have happened in our injections. We endeavored to minimize it by flooding the muscles with ink, as described above, and also to reduce its effect by taking the averages of counts from a great many fields from each preparation. On the basis of our rather extensive experience we doubt that it occurs on a large enough scale to impair the validity of results obtained by the ink injection method.

Fasciculi and muscle fibers. In the gracilis muscle of the dog the fibers are grouped in well marked fasciculi, usually triangular in cross section in

fixed preparations. The average number of fibers in 140 fasciculi selected at random was 66. No fasciculi were observed with fewer than 30 fibers and none with more than 125. The mode of the distribution curve was at 60 fibers, and sixty per cent of the fasciculi contained between 45 and 70 fibers. A number 10 ocular and 4 mm. objective were used, the former equipped with a micrometer. Fiber counts were not made in the early experiments (those by E. W.). In the later experiments (by M. M.) in which fibers were counted the micrometer field used was a rectangle 69 microns long and 34.5 microns wide. In two hundred such fields from one muscle the average number of fibers per field was 4.12. In only two fields were there fewer than three fibers in the field, and in only fourteen were there more than five fibers in the field: ninety-two of the two hundred fields contained four fibers. This uniformity of cross section of fibers is fully as great as one would expect to find in view of the fact that some of the fields are bound to contain fibers sectioned near their ends, where they have begun to taper. The average cross sectional area of these fibers was 0.58×10^{-3} sq. mm., and the average diameter 28 microns. The dog from which this muscle was taken was full grown, weighing about 12 kgm. Another muscle, from a dog weighing 21 kgm., was composed of considerably larger fibers, the average cross section of these being 0.77×1^{-3} sq. mm., and their average diameter 32 microns. In a third dog, a puppy weighing 6 kgm., the average fiber cross section was only 0.44×1^{-3} sq. mm., and the average diameter only 24 microns. There was also considerably greater variation in size of fibers in this muscle from a puppy than in the muscles obtained from full grown animals. Fiber counts were made on six gracilis muscles in addition to the three described above; the values from all six being intermediate between the second and third of those described. In terms of number of fibers per square millimeter of cross section our figures for gracilis muscles from full grown dogs range from 1291 to 2012, averaging 1640. This latter average implies an average cross section of individual fibers of 0.61×10^{-3} sq. mm. and an average fiber diameter of 29 microns.

It should perhaps be pointed out that so far as these measurements may be in error the error is almost certainly in the direction of greater apparent fiber cross section, and hence of fewer fibers per sq. mm. of muscle cross section than exist in the intact organism. The fixative used by us (Bouin's fluid) is stated by experienced histologists not to induce shrinkage of muscle tissue, and we saw no evidence of such shrinkage in our preparations. Since our muscles were fixed under less tension than prevails in situ (see p. 408) the only possible departure, therefore, from the dimensions of the intact muscle is in the direction of fiber thickening.

The capillary supply of the gracilis muscle. Following the example of Krogh (1919) we injected the gracilis muscle in one dog after dilating the

vessels with amyl nitrate. This dog, weighing 21 kgm., was anesthetized with "nembutal" (sodium ethyl (1 methyl-butyl) barbiturate). It showed an arterial pressure, prior to administration of amyl nitrite, of 134 mm. Hg. A small quantity of the latter drug was introduced intravenously, whereupon the arterial pressure fell abruptly to 18 mm. Hg. Shortly after this level was reached ink was injected according to the method described near the beginning of this paper. The muscle became very black, and when sectioned showed a very uniform distribution of capillaries. The average number per sq. mm. of cross section was 2580, thus agreeing closely with the findings of Krogh on the semimembranosus of the dog. The average number of muscle fibers per sq. mm. of cross section in this muscle was 1291, consequently there were two capillaries, on the average, for each muscle fiber. As a check on this ratio, which seems to us important, we compared the ratios of capillaries to muscle fibers in two microphotographs of injected horse muscle published by Krogh. In one of these (1919) the ratio is approximately 2.6, and in the other (1922) approximately 2.0. Paff (1930) using a standard injection mass, instead of india ink, obtained in a series of eight muscles from rat, guinea pig and cat an average ratio of fibers to capillaries of 2.6 (calculated by us from his table). In four of his eight (including all three of his cat muscles) the ratio ranged between 1.81 and 1.92. We were able to obtain a microphotograph of a particularly heavily injected area from the gracilis on which our counts were made, and this area showed a ratio of approximately 2.5. We thus have a fairly definite picture of the potential blood supply of individual muscle fibers in the gracilis muscle, the usual distribution being of the order of two capillaries to the fiber, and two and a half to the fiber the approximate maximum in the most heavily vascularized areas.

Capillary counts in resting muscles. One gracilis muscle was injected with india ink in each of eight full grown dogs, as early as possible after complete anesthetiziation, and with the animals quiet. In five of the experiments amytal was used as the anesthetic, in two morphia and ether, and in the remaining one, decerebration under transient etherization.

A striking observation, and one that seems to us probably significant, is that in all these muscles fasciculi that showed no injected capillaries, or at most a very few, were interspersed among well-injected fasciculi. The fasciculi in which the capillaries were devoid of ink were practically always penetrated by larger vessels in which ink was present, hence the failure of these capillaries to take ink cannot be accounted for on the assumption that they sprang from a different arterial source. Nor would the well-known richness of anastomosis among the intramuscular vessels justify such an assumption. In one muscle, which we examined with some care, approximately sixty per cent of the fasciculi showed characteristic injection of capillaries and forty per cent absence of capillary injection. The obvi-

ous suggestion is that the rotation of function among the capillaries postulated by Krogh (1919) operates in terms of whole fasciculi, which alternate in receiving the blood-flow characteristic of resting muscles and in being virtually deprived of blood for a time.

Capillary counts were made in injected areas, and in six of the eight muscles under discussion these were very consistent, ranging between averages of 917 and 1154 per sq. mm. for individual muscles, with an average for the group of 1056 per sq. mm. The other two muscles (the first two of the series) showed markedly fewer capillaries, each averaging 770 per sq. mm. We consider the consistent showing of the group of six muscles to be probably more nearly characteristic of gracilis muscles in general, than the smaller figures, and consequently look upon the value 1050 as the most probable approximate average number of open capillaries per square millimeter in such fasciculi of resting gracilis muscles as are receiving blood. The average number of muscle fibers per sq. mm. is stated above (p. 409) to be 1640, hence the ratio of open capillaries to fibers is 0.64. The puppy on which observations were made (p. 409) showed an average of 1690 capillaries per sq. mm. cross section, a value more than 50 per cent greater than the average given above. Its muscle fibers were smaller, however, than the corresponding fibers in full grown dogs, consequently the ratio of open capillaries to fibers was less disproportionate, being 0.74, as compared with 0.64 for the full grown animals.

Capillary counts in muscles injected during exercise. In each of eight dogs one gracilis muscle was prepared for injection in the manner described, and then stimulated rhythmically through the obturator nerve by brief tetanic shocks repeated about once a second. Stimulation was continued for three to five minutes to give time for full adjustment of the intramuscular circulation to the exercise state. Ink was then injected *while the exercise was still in progress,* and the subsequent procedures carried out as described. The capillary counts were reasonably consistent, the averages from the different muscles ranging from 1708 to 2437 per sq. mm., with an average for the series of 2010 per sq. mm. The ratio of this average to the average number of fibers per sq. mm., 1640, is 1.23, which is approximately twice the ratio found in resting muscles. It is, however, well below the ratio found in muscles treated with amyl nitrite (p. 410), so that while exercise by direct stimulation about doubles the number of open capillaries in individual areas it does not appear to cause all that are present to open, or at least to open widely enough to take ink.

Even in these exercised muscles there were occasional fasciculi with uninjected capillaries interspersed among the fasciculi that had taken ink in characteristic fashion. The proportion was much smaller, however, than in resting muscles. In an effort to discover whether the proportion of fasciculi with open capillaries increases progressively as the exercise pro-

ceeds, as well as to discover whether the increase in number of open capillaries in other fasciculi is also progressive during exercise, two more muscles were injected, one after exercise had been under way for about one minute, the other as soon as convenient after stimulation was begun, probably about 15 to 20 seconds after the first contraction. The ratio of capillaries to fibers was 1.12 in the first of these and 0.91 in the second, thus indicating quite clearly that the capillaries open progressively during the early stages of exercise. A careful estimate of the proportion of fasciculi with uninjected capillaries was made in the second of these two muscles. Approximately thirty per cent of the fasciculi were in this state, indicating that the proportion of fasciculi receiving blood during exercise also increases progressively.

Capillary counts in muscles injected late in experiments and under different arterial pressures. It was a matter of interest to discover whether a definite relationship exists between general arterial pressure and the number of open capillaries per sq. mm. of muscle cross section. The experiments thus far described failed to throw light on this question because the arterial pressures did not vary materially. They approximated 100mm. Hg in all the significant experiments, in no case falling below 90 mm. Hg, nor exceeding 120 mm. Hg. We have a significant series, however, consisting of six injected muscles from animals that had been subjected to drastic experimental treatment and had been under the influence of amytal for at least an hour and a half. Two of these animals had been curarized; one had had both phrenic nerves cut; the remaining three had had vigorous artificially induced exercise of all the main muscles for fifteen minutes followed by a recovery period of at least an hour. The arterial pressures and capillary counts are given in the subjoined table.

ARTERIAL PRESSURES	CAPILLARY COUNTS PER SQUARE MILLIMETER
35	1,496
38	1,877
60	1,851
80	1,903
137	1,703
142	1,782

With a single exception the counts fall within a narrow range although the differences in arterial pressure are extreme. We see no indication that arterial pressure as such is a significant determinant of the capillary count. Either the prolonged anesthetization or the treatment to which the animals were subjected, or both in combination operated, however, to cause capillaries to open to an extent not materially below that induced by exercise.

Capillary counts in muscles injected post-mortem. We considered it of

interest to determine whether the capillary counts in muscles injected post mortem without previous dilatation with amyl nitrite would approximate more nearly the counts in muscles injected during exercise or in muscles injected after amyl nitrite. Accordingly both gracilis muscles were prepared for injection in each of three dogs. Instead of injecting ink into the blood stream immediately, however, the femoral artery on one side was tied above the origin of the gracilis artery, and the vessels of the muscle flushed out with Ringer's fluid, which was introduced under moderate pressure through the cannula which had previously been placed in position for making the ink injection. Washing with Ringer's fluid was continued until no red color could be detected in the fluid escaping through the vein. The muscle was then injected with undiluted india ink under moderate pressure. The interval between the stoppage of the circulation and the injection of ink was about a half hour. The same procedure was followed on the opposite side of the body except that as soon as the flushing with Ringer's fluid was completed the muscle with its connecting vessels, and with the cannula in position, was dissected out and placed on ice for 24 hours, and was then injected with ink.

The average capillary counts for the three muscles injected one-half hour after stoppage of the circulation were 1981, 2266, and 2310, the mean being 2185, and for the three muscles injected after twenty-four hours on ice, 1730, 1756, and 1877, the mean being 1790. It was noteworthy that no uninjected fasciculi were seen in any of these muscles, the distribution of capillaries being very uniform over all parts of the sections.

It will be noted that the capillary counts from these muscles approximate more nearly the counts from muscles injected during exercise than those from muscles after amyl nitrite. The possible significance of this finding will be considered in the discussion.

Observations on semimembranosus muscles. In a 16 kgm. dog, anesthetized with "nembutal," and with all muscles at rest the semimembranosus muscle on one side was injected with ink, the arterial pressure at the moment of injection being 96 mm. Hg. Amyl nitrite was then introduced intravenously. The arterial pressure fell to 40 mm. Hg, and at this pressure the semimembranosus on the opposite side was injected with ink. The injection technique was similar to that described for the gracilis, the main artery to the muscle being flooded with ink from a side branch, after the vessels to other muscles than the one to be injected had been tied.

To our surprise the fibers of this muscle proved to average much smaller than in any of the gracilis muscles examined. The fibers of the first muscle averaged approximately 4400 per sq. mm. of cross section; corresponding to an average fiber cross section of about 0.23×10^{-3} sq. mm., and an average fiber diameter of 17 microns. The fibers of the second muscle averaged approximately 3800 per sq. mm. of cross section, corresponding to an aver-

age fiber cross section of about 0.26×10^{-3} sq. mm., and an average fiber diameter of 18 microns.

The capillary counts were reasonably consistent with expectations based on our observations on gracilis muscles. Thus, the average count in the resting semimembranosus was 3240 per sq. mm., the ratio to fiber count being 0.74, as compared with an average of 0.64 in gracilis muscles under corresponding conditions. This muscle showed an alternation of injected with uninjected areas, similar in general to the alternation seen in resting gracilis muscles, although there was much less uniformity in the distribution of injected areas in relation to uninjected, a uniformity which was usually quite marked over the whole of the resting gracilis. The average count of capillaries in the muscle injected after amyl nitrite was 5900 per sq. mm. The ratio to fiber count in this case was 1.55, considerably smaller than the corresponding ratio in the gracilis, but still indicating a more extensive opening of capillaries than we have observed under any treatment other than amyl nitrite. The fact that our average capillary count is so much higher than Krogh's on a corresponding muscle we attribute to an individual variation, since if the fibers in this muscle had been of average size, and the capillary-fiber ratio unchanged, the capillary count would have agreed satisfactorily with that reported by Krogh.

Discussion. The observations on resting muscle reported in this paper appear to us to support the conception of the capillary circulation advanced by Krogh except for the detail that it indicates the blood supply to such parts of the resting muscle as are receiving blood to be much more copious than was suggested in his postulate. In this respect our findings corroborate those of Hartman, Evans and Walker on cats' muscles (1929). The fact that every resting muscle examined by us showed areas devoid of open capillaries interspersed among areas with open capillaries can best be explained in terms of Krogh's idea of a rotation of function among the capillaries. The observation that the fasciculi appear to be the circulatory units in that they tend either to show the blood supply characteristic of resting muscles or to be practically devoid of blood in their capillaries, is an interesting anatomical detail which does not appear to us at the moment to have any special significance.

We desire to call particular attention to the fact that under four quite diverse conditions, i.e., muscular exercise (after adjustment of the circulation has had time to take place), in the last stages of drastic and prolonged experimentation upon the entire animal, soon after washing the blood out of the vessels of the muscle and probably before actual death of the muscle cells, and finally, after excision and exposure to cold for twenty-four hours, the capillary counts fall consistently within a fairly narrow range, of which the mean value is virtually twice the mean capillary count in blood-receiving areas of resting muscle. This consistency seems to us to be too marked

to be accidental. It is most simply explained as merely the result of relaxation of the walls of all the capillaries to what might be termed a resting position, or at least one in which constrictor influences are absent. In other words, we offer the suggestion that the dilatation of the capillaries observed under the four conditions described is due to the abatement of influences which tend to cause capillary constriction, rather than to the onset of positive capillo-dilator influences. A definite argument in favor of this idea is that it is obviously the most likely explanation in the case of muscles injected post mortem.

If this suggestion is to merit serious consideration it must account for the fact that after treatment with nitrites a considerably larger number of capillaries can be injected than under any of the above conditions. In explanation we would revive an idea voiced some years ago by Cohnstein and Zuntz (1888), that every capillary field contains numbers of capillaries whose cross-section, even in the relaxed state, is so small that they will not permit corpuscles to pass, and hence, as Krogh showed (1919), will not take ink. Our data do not support the idea of these authors that they open out when the pressure becomes high. Under the action of a powerful capillary dilator like amyl nitrite we presume them, however, to dilate beyond their "resting" state, thus acquiring a sufficient caliber so that they can be injected. This suggestion carries the implication that these particularly small capillaries will never be traversed by corpuscles under "physiological" conditions. They may or may not be closed completely under circumstances in which the majority of capillaries are in a semiconstricted state, but their function can not extend beyond that of transporting plasma, even when in a state of "normal" dilatation.

In his extensive discussion of capillary function, Hooker (1921) cites observations which suggest strongly that individual capillaries take on a greater caliber under the influence of powerful capillary dilators than they can be caused to take on by any other means. These observations harmonize with our idea that the exceptionally high capillary counts after nitrites are due to the resultant widening of capillaries that are normally too narrow, even when free from constrictor influence of any sort, to take either red corpuscles or ink.

SUMMARY

1. In the resting gracilis muscles of dogs the fasciculi appear to be the circulatory units in the sense that in the "rotation of function" among the capillaries, typical of skeletal muscles, the capillaries of individual fasciculi tend either to be closed en masse or to be open to an extent characteristic of muscles in a state of rest. When so open the ratio of number of open capillaries to number of muscle fibers averages about 0.64. Capillaries are designated as open when they will take india ink upon mass injection of ink into the vessels of the muscle.

2. Under the following conditions, i.e., during exercise, toward the end of long and drastic experiments on the entire animal, and both early and late after stoppage of the circulation, the number of open capillaries averages about double the number in injected fasciculi of resting muscle. A few uninjected fasciculi are seen in muscles injected after four to five minutes of exercise, none in muscles under the other conditions described.

3. The marked agreement among the capillary counts under the diverse conditions cited in the above paragraph is interpreted to mean a general relaxation of all the capillaries to a "resting" state. The abatement of influences tending to cause capillary constriction is considered to account for this relaxation rather than the onset of positive capillo-dilator influences.

4. After administration of amyl nitrite the ratio of number of open capillaries to number of muscle fibers ranges between 2 and 2.5, as compared with about 1.2 for the corresponding ratio under the conditions cited in paragraph 2. To explain this marked increase in number of open capillaries an old assumption is revived, to the effect that a considerable number of capillaries are present in addition to those that can be injected under the conditions noted in paragraph 2, these additional capillaries being of such small caliber that they cannot be injected even when normally relaxed, but under the influence of powerful capillo-dilators will relax still further, to a caliber at which they will take ink.

5. In terms of number of units per sq. mm. of cross section the following are the average data for gracilis muscles of the dog, as reported in this paper. Muscle fibers, 1640; open capillaries after amyl nitrite, 2580; in injected areas of resting muscle, 1050; during muscular exercise, 2010; in the late stages of long experiments, 1820; a half hour after replacing the blood with Ringer's fluid, 2185; twenty-four hours after excision of the muscle, 1790.

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REFLEX STIMULATION OF RESPIRATION FROM INCREASE IN VENOUS PRESSURE

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During some studies on cardiac dyspnea it was found that persons with cardiac disease reacted to muscular exercise with a greater increase in ventilation than did normal subjects. As neither group of individuals exhibited alterations in the composition of the blood (Cullen, Harrison, Calhoun, Wilkins and Tims) it seemed that the cause of this phenomenon had to be sought elsewhere. Schott has shown that muscular effort causes a greater and more lasting rise in the venous pressure of such patients than in that of normal subjects. Bainbridge demonstrated that rise in venous pressure causes reflex acceleration of the heart. It seemed possible that there might be a similar reflex effect on respiration and the present study was made in order to test this hypothesis.

Our observations were made upon dogs anesthetized with barbital. Respirations were recorded with a Benedict spirometer. In the first group of observations fluid—either Ringer's solution, normal saline solution, or defibrinated blood—was injected rapidly into the external jugular vein or into the inferior vena cava. Examples of such experiments are shown in figure 1. Well marked increases in respiratory rate and in minute ventilation resulted when the vagus nerves were intact. After bilateral vagotomy, no change or only slight increase in respiration was observed when fluid was injected.

In order to determine whether the acceleration of breathing was related or not to a temporary plethora *per se*, other experiments were done in which fluid was injected into the external jugular vein low in the neck, or the inferior vena cava high in the abdomen, the cannula pointing away from the heart. By this means it was possible to produce plethora and increase in pressure in large veins without causing increase in pressure in the right auricle or in the veins adjacent to it. Under such conditions injection of similar amounts of fluid was entirely without effect on respiration.

A second group of experiments was then performed. A rubber balloon, improvised from a rubber catheter and a condom, was passed into the right auricle through either of the veins already mentioned. Air could be injected into or withdrawn from the balloon at will. The results of such an

experiment are shown in figure 2. When as little as twenty cubic centimeters of air were introduced into the balloon there was a progressive increase in respiratory rate. Deflation of the balloon caused the reverse change. After bilateral vagotomy similar but much less striking changes were noted in some dogs while in others no effect was observed. When the vagus nerves were intact these changes occurred in every instance.

These experiments seem to indicate that elevation of the venous or intra-auricular pressure causes reflex stimulation of breathing, the reflex arising

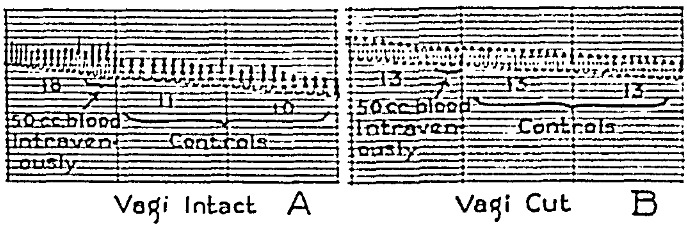

Fig. 1. The curve runs from right to left

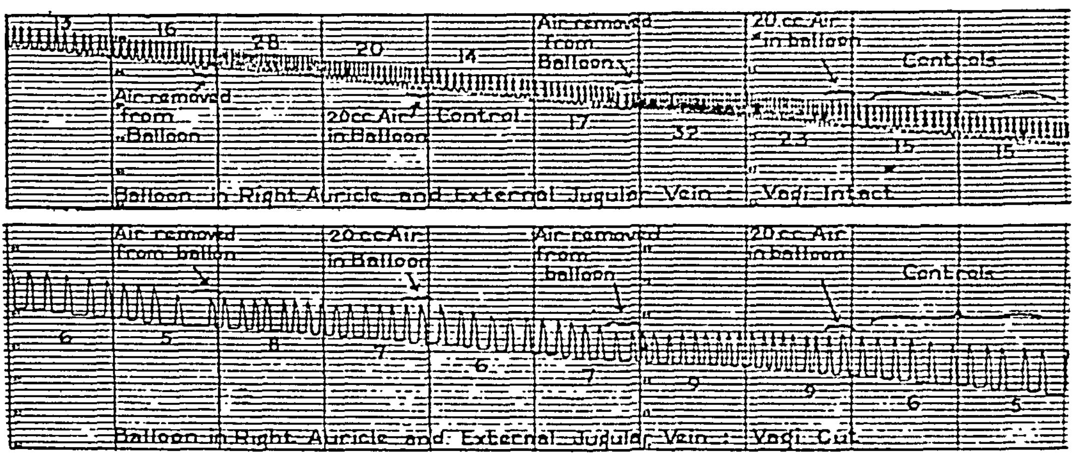

Fig. 2. The curve runs from right to left

either in the heart or in the great veins very close to the heart, and the vagus nerves being the main afferent path. The observations are in accord with those of Heymans and Heymans who observed, in cross circulation experiments, that changes in the pressure of the blood in the heart caused reflex changes in breathing. Somewhat similar findings were reported by Sutton and Lueth who found that distention of the left ventricle produced dyspnea in the dog. It seems likely that both of the experiments reported by these authors and also our observations are of some importance in explaining the respiratory response to muscular exertion, and

especially so in regard to the abnormally great increase in ventilation which occurs during exercise in persons with cardiac disease.

SUMMARY

Increase in the venous pressure produced either by rapid intravenous injection of fluid, or by distention of a balloon in the right auricle is accompanied by increase in the respiratory rate and in the ventilation of dogs with intact vagi. The same procedures were usually without effect after double vagotomy.

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HUMAN MILK STUDIES

XI. Vitamin G (B_2) Content of Mixed Milk[1,2]

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Clinical observations (1–4) and animal experimentations (5–8) indicate that both mother's and cow's milk have a relatively low potency in the vitamin B complex[3] and at times may be insufficient to satisfy the vitamin demands of the growing young when milk is the sole food supply. The vitamin B complex content of breast milk is dependent on the richness of the maternal dietary in this vitamin moiety and on the extent to which it is used up in the ordinary maternal bodily functions and in the additional processes of milk secretion.

Since human milk is the natural food for the infant during the first months of life, its evaluation in the known dietary components is of importance. With a knowledge of the amounts of the various food principles in mother's milk, it is possible to provide the young with a diet adequate for growth and well-being, since the necessary supplements can then be provided either by direct administration to the child's diet or by reinforcing the milk through the maternal dietary (9–11). Adequate nourishment during early life is one of the fundamentals in the achievement of good bodily structure and a stable functional capacity.

[1] This study was kindly aided by a grant from the Committee on Scientific Research of the American Medical Association. A preliminary report was presented before the Division of Biological Chemistry at the 82nd meeting of the American Chemical Society in Buffalo, August 31 to September 4, 1931.

[2] We wish to express our thanks and appreciation to Maurice H. Givens, Ph.D., of the Northwestern Yeast Company, Chicago, for the yeast used in this study, and to Arthur D. Holmes, Ph.D., of the E. L. Patch Company, Boston, for the cod liver oil. It is a pleasure to acknowledge the continuous interest and coöperation of the Mother's Milk Bureau of Detroit.

[3] In this report the old vitamin B that is now known to be composed of several fractions will be referred to as the vitamin B complex; the antineuritic factor will be spoken of as vitamin B (B_1); and the so-called antipellagra factor as vitamin G (B_2).

In these laboratories, a flattening of the growth curve was noted in experimental rats receiving an adequate diet with human milk as the only source of the vitamin B complex (5). The addition of autoclaved yeast stimulated some renewed growth, indicating that mother's milk in the amounts fed, contained insufficient vitamin G, or other factors present in yeast so treated, to sustain growth. An even greater response was produced by supplementing the diet with whole untreated yeast which bears evidence that both the antineuritic vitamin B and vitamin G, as well as well as other possible vitamin fractions present in yeast, may be limiting factors in the adequacy of mother's milk. By adding 10 grams of whole dried brewer's yeast daily to the diets of lactating women, the individual milks became more effective as a source of the vitamin B complex (11). Experimental rats, receiving milk from the mothers who had taken yeast, grew at an accelerated rate on less food. A clinical counterpart in the human infant has been reported by Hess (12) who gave whole brewer's yeast directly to infants; likewise there was a gain in weight which the author explains, "was due no doubt, in some cases to the stimulation of appetite, but in others there was a prompt response although the total intake of food remained the same." It is an indication that some factor in yeast which effects a more efficient utilization of food by the infant is the same as that conferred to the milk of mothers taking yeast.

The occurrence of borderline deficiencies in infants (1–4) makes important the quantitative determination of each of the vitamin fractions in milk. Cow's milk has been found to be richer in vitamin G than the antineuritic vitamin B (13–14). The following note on the vitamin G concentration of pooled breast milk is preliminary to more extensive studies on the potency of milk as it is influenced by vitamin additions to the human maternal dietary.

Experimental procedure. The method followed in determining the vitamin G content of human milk was essentially that developed by Bourquin and Sherman (16). A unit of vitamin G is taken as the amount of test substance necessary to cause an average weekly gain of 3 grams in standardized test rats over a period of 8 weeks, the animals having been depleted previously of any vitamin storage by retaining them on a vitamin G-free diet for a period of two weeks or more.

The basal vitamin G-free diet was fed *ad libitum* and consisted of 18 grams casein,[4] 68 grams dextrin,[5] 4 grams Osborne and Mendel salt mixture, 8 grams filtered butter fat, and 2 grams cod liver oil. Apart from

[4] The casein was treated with acidulated water (16), then extracted with 60 per cent alcohol (17) followed by washing with 95 per cent alcohol.

[5] An 80 per cent alcoholic extract of whole wheat equivalent to 50 grams of untreated wheat was dried upon 18.75 grams of the dextrin.

the basal diet, graded quantities of fresh pooled human milk[6] and 2 drops of wheat germ oil[7] were fed daily.

The positive control rats received the same diet as the experimental animals on milk with the exception that the source of vitamin G was alkaline autoclaved yeast instead of milk. Since yeasts vary widely in the potency of both vitamins B and G (18, 19), it is necessary to know the original potency of samples to be used in experimental studies as well as the amount of destruction of the various factors by different methods of treatment.

By preliminary tests, it was demonstrated that the yeast used in this study was rich in both factors. One sample was autoclaved for a period of from 3 to 6 hours at a pH of approximately 4.5 and another sample in an alkaline medium to note the degree of destruction of the vitamins by the various types of treatment. The results showed that the yeast that had been moistened with tenth normal NaOH and autoclaved for 6 hours at 15 pounds pressure (18) had a more complete destruction of vitamin B than did the samples autoclaved at a pH of approximately 4.5 for the same length of time. For this reason, the alkalized autoclaved yeast was used as a source of vitamin G for the positive control animals in this study. Such severe treatment of the yeast, however, caused some decrease of vitamin G also, as was shown by lessened rate of growth in the test animals. This is in accord with the findings of other investigators (20–22).

Results and discussion. The growth of the standardized test rats fed daily quantities of 3 cc., 5 cc., 10 cc., 15 cc., and 20 cc. of fresh pooled human milk as the only source of vitamin G in a diet otherwise adequate is given in chart 1. A group of animals on the vitamin G-free basal ration was observed as negative controls and another on the same diet plus 0.40 gram alkalized autoclaved yeast daily, served as the positive controls.

The results demonstrate that as the supply of milk was increased, the growth rate of the animals likewise increased. The mean increments in weight of the rats for the 8 weeks' experimental period on the various levels of milk were as follows: 3 cc. gave 19 ± 3.0 grams, 5 cc. gave 32 ± 2.6 grams, 10 cc. gave 41 ± 2.4 grams, 15 cc. gave 72 ± 3.7 grams, and 20 cc. gave 94 ± 1.2 grams. But even at the 20 cc. level, the highest amount of milk fed, the growth of the animals did not approximate that of the positive controls which made gains of 154 ± 1.2 grams.

The negative control rats showed varying degrees of vitamin G de-

[6] The mothers contributing milk for this study were American women coming from average homes. They chose their diet unrestrictedly except that no supplementary additions of yeast were permitted. Each produced enough milk to feed her own infant as well as furnish 20 to 25 ounces each day to the Mother's Milk Bureau of Detroit.

[7] An anhydrous ether extract of wheat germ.

ficiency. There was a definite stasis in growth within two to four weeks after the experimental diet was instituted. These negative controls de-

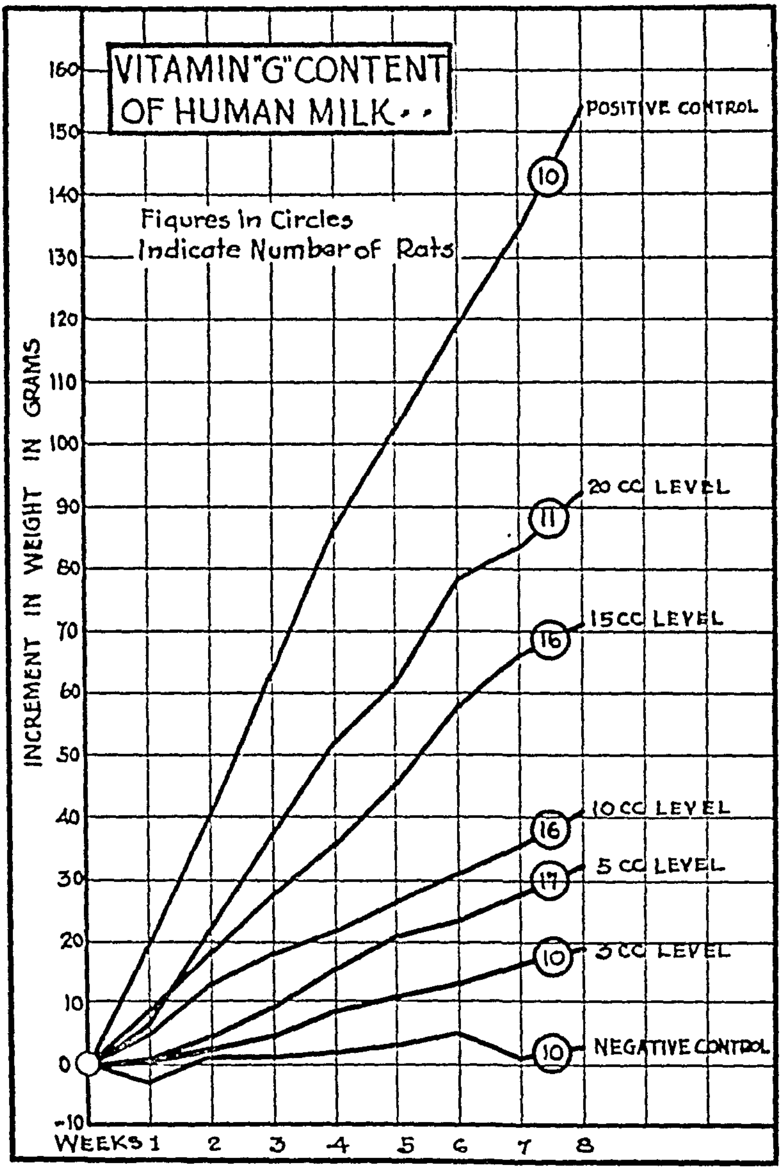

Chart 1. Illustrates the growth curves of the standardized test rats. The positive controls received the vitamin G-deficient basal diet plus 0.4 gram alkalized autoclaved yeast daily as the source of vitamin G; the negative controls received the basal diet without supplements; the experimental rats received adjuvants of 3 cc., 5 cc., 10 cc., 15 cc., and 20 cc. of fresh pooled breast milk daily as the sole source of vitamin G for a period of 8 weeks. Figures in the circles indicate the number of animals in each group from which the composite growth curves are computed.

veloped an unkempt appearance and the tails, ears, and medial portions of the legs became encrusted with a waxy, sebaceous deposit. Alopecia

was common, particularly around the eyes; pronounced pellagra-like lesions were not always present, but in all animals on a vitamin G-deficient diet there was an inflammatory condition of the forepaws and nose with evidence of abrasions.

In the present study, the unit rate (15) of growth of the rats was attained by feeding 3 cc. and 5 cc. quantities of milk per day per rat. Although these quantities of mixed human milk were sufficient to support some gain in body weight of the experimental rats, deficiency symptoms appeared in 70 per cent of the animals receiving 3 cc. of milk, and 59 per cent of those getting 5 cc. each day.

The final weights of the animals receiving smaller amounts of milk were more variable than those getting larger quantities. It is noted that for the daily intake of 3 cc. and 5 cc. of breast milk, the coefficients of variability were 28 and 25 respectively in contrast to 13, 18, and 6 for the 10 cc., 15 cc., and 20 cc. respectively.

The depletion of yet unidentified dietary essentials can not be cited as the causal factor of the greater variability among groups getting less milk, for, as expressed by Sherman (15, 19), these factors are conserved by the slow growth rate obtained when small quantities of the test vitamin are fed. Thus, in these observations on the lower levels of milk, the greater variability may be attributed to the lack of vitamin G and the accompanying lowered physical state.

In connection with this study, it is of interest to review the observations recorded by Guha (23) in which he reports that experimental rats that had reached a stasis in growth on a diet containing vitamin B in the form of a Fuller's earth preparation of wheat germ and vitamin G as an alkaline autoclaved marmite were stimulated to resume growth by the addition of cow's milk to the diet. In these laboratories, when breast milk was fed to standardized rats as the only source of the vitamin B complex in an otherwise complete diet, autoclaved yeast supplements stimulated a resumption in growth. It would appear then that the undetermined dietary factor described by Guha is present in breast milk and that it is not a limiting factor in the studies recorded herein.

SUMMARY

Under the conditions of these experiments, fresh mixed human milk in quantities from 3 cc. to 5 cc. daily as the only source of vitamin G in a diet otherwise complete, was sufficient to support the unit gain in weight of 3 grams weekly in young standardized test rats throughout the experimental period of 8 weeks. The greater number of animals, however, on these levels of milk showed deficiency symptoms. Quantities of 10 cc., 15 cc., and 20 cc. daily produced increasing gains in body weight, although none attained the rate of growth established by the positive controls.

If the above facts on breast milk produced by mothers on dietaries which are known to be adequate can be applied to infant feeding on the basis of comparative body weights, the quality of breast milk in some cases where the maternal diet is limited, either through choice or deprivation, may fall short of satisfying the vitamin G needs of the rapidly growing infant.

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THE VITAL CAPACITY OF THE LUNGS AT LOW BAROMETIC PRESSURE.

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Authorities have for a long time agreed that a decrease in the vital capacity of the lungs takes place when one is first exposed to a low barometric pressure. Of these the earliest record is that of Paul Bert (1878) who noted a diminution of as much as 50 per cent in the vital capacity at the summit of Monte Rosa.

The change in vital capacity seems to be without practical importance in the adaptations for obtaining oxygen and may be, therefore, of only theoretical interest. The writer having recently had occasion to review the literature on the subject was stimulated to delve into experimental data he has accumulated from time to time over a period of 19 years. Almost every group of physiologists that undertakes an experimental expedition into the mountains for the study of acclimatization sooner or later makes observations on the vital capacity. The last to report on a series of tests was Grollman, in 1930, who observed a decrease of 11.2 per cent in himself while on Pike's Peak.

Various theories have been advanced to account for the decrease. Zuntz and his co-workers (1906) attributed it to an expansion of intestinal gases; Durig (1909) looked upon fatigue of the respiratory muscles as the explanation; Fuchs (1908) regarded it as a result of increased muscular tone due to the low temperatures at which the subjects lived; and Mosso (1889) argued that the blood collects in the lungs during inspiration in excess of the amount expelled by the following expiration, thus leaving less space for air. Since most of the bodily changes observed at high altitudes can be directly or indirectly attributed to the relative shortage of oxygen there experienced, it is here our purpose to inquire whether the decrease in vital capacity may not be, at least in part, due to anoxemia.

Observations in a low pressure chamber. During a period of two years data on the vital capacity were accumulated from observations made on 24 men while exposed to low barometric pressures in a low pressure chamber. Only a part of the data is given here. It was our custom to lower

the pressure by stages and to maintain a constant pressure at each step for from 10 to 15 minutes. We ordinarily used simulated altitudes of from 18,000 or 20,000 feet and occasionally as high as 25,000 feet. We invariably secured some reduction in the vital capacity, the average decrease at 20,000 feet was 350 cc., approximately 8 per cent; while the maximum for one man at that altitude was 1080 cc., or a 25 per cent reduction. There was considerable individual variation in the amount of reduction in atmospheric pressure required to induce a decrease in vital capacity. No one gave a definite response at less than a simulated altitude of 10,000 feet, while the break for most of our subjects came at altitudes of from 12,000 to 15,000 feet. The breaking point of two men is shown in table 1. In these cases the step upward in simulated altitude was by units of 2000 feet each. The run was up to 20,000 feet and then by steps back to sea-level. One of these men experienced the change at about 14,000 and the other at around 18,000 feet.

TABLE 1

A study to determine the "point of break" in the vital capacity of 2 subjects at simulated altitudes

Vital capacity in liters

SEA LEVEL	6,000 FEET	8,000 FEET	10,000 FEET	12,000 FEET	14,000 FEET	16,000 FEET	18,000 FEET	20,000 FEET	18,000 FEET	16,000 FEET	14,000 FEET	12 000 FEET	10,000 FEET	SEA LEVEL
4.85	4.85	4.85	4.85	4.86	4.80	4.86	4.73	4.55	4.70	4.75	4.81	4.80	4.91	4.89
4.72	4.76	4.85	4.79	4.75	4.62	4.55	4.35	4.35	4.40	4.57	4.65	4.73	4.74	4.89

Table 2 presents three series of observations made in a study of the effects of the administration of pure oxygen. There were seven men who served as subjects, the data for only three of these are given in the table. During exposure to lowered barometric pressure without access to extra oxygen, all of the men showed a reduction in vital capacity. In the second series of runs each man received a large and steady flow of oxygen throughout the entire period of exposure to the reduced barometric pressure. The oxygen was delivered through a rubber tube that was held in the mouth like a pipe stem. The average decrease in vital capacity for the group of 7 cases at the simulated altitude of 20,000 feet was 360 cc. without oxygen and 130 cc. when oxygen was supplied throughout the run. It is evident, therefore, that oxygen may in large part prevent the decrease in vital capacity. Only one man, however, was completely saved from this effect by the oxygen (see T. V. in table 2).

In the third series of runs of which three examples are given in table 2, the men went without an extra supply of oxygen until they reached the simulated altitude of 20,000 feet. After 10 minutes of exposure to this

pressure, they were given oxygen in the usual way. The average decrease in vital capacity for the group of seven men was 510 cc. when 20,000 feet was reached. The breathing of the extra oxygen raised the average capacity from 3940 cc. to 4230 cc., which was only 220 cc. below the normal, 4450 cc., for the group. The return to normal was complete in three of the men, while a fourth gained nothing by breathing the extra supply of oxygen.

The method of administering oxygen was wasteful and undoubtedly did not raise the alveolar oxygen tension of all the subjects in equal degree.

TABLE 2

Vital capacity without O_2 during exposure to low barometric pressure, while taking O_2 throughout the run, and as a result of taking O_2 at the lowest pressure

Vital capacity in liters

SUBJECT	STIMULATED ALTITUDE						
	0 feet	10,000 feet	15,000 feet	18,000 feet	20,000 feet	22,000 feet	25,000 feet
	Without oxygen						
P. B.	4.50	4.32	4.20	4.14	3.95		
S. F.	4 42	4.40	4.40	4.05	3.85		
T. V.	4.35	4.30	4.30	4.25	4.15		
	Taking O_2 from the start						
P. B.	4.40	4.36	4.24	4.40	4.33	4.32	4.19
S. F.	4.38	4.30	4.15	4.13	4.11	4.10	4.01
T. V.	4.39	4.31	4.29	4.21	4.34	4.30	4.33
	Without O_2 and then administration of O_2 at 20,000 feet, lowest pressure						
						20,000 feet	
P. B.	4.50	4.32	4.15	4.00	3.95	*4.05*	
S. F.	4.32	4.30	4.43	4.00	3.98	*4.21*	
T. V.	4.75	4.68	4.48	4.42	4.40	*4.80*	

It is unfortunate that we made no analyses of the alveolar air for oxygen. Our results indicate that the decrease in vital capacity during exposure to a low barometric pressure is due to anoxemia, and that it may be wholly, or at least in part, prevented by the administration of oxygen.

That the decrease in vital capacity is not due to the expansion of intestinal gases was indicated in the "come back" of our cases when oxygen was administered. Breathing an extra supply of oxygen would in no way affect the gas volume in the intestine. We measured the circumference of the abdomen of certain of our subjects and found some had a noticeable expansion without a marked reduction in vital capacity, while others had a

marked reduction in capacity without any external evidence of the presence of intestinal gases.

Observations on Pike's Peak. During three expeditions to the summit of the Peak (altitude 14,110 feet) the change in vital capacity was studied on nine men; two of the men were under observation during two, and a third during three, sojourns on the mountain. The data for two of the expeditions are summarized in table 3. It was our custom to allow three trials each time a man was tested. The data in the table give the largest volume obtained each day.

The vital capacity of every man was at first distinctly decreased by the ascent of Pike's Peak, this decrease ranging from 6.7 to 15.3 per cent.

TABLE 3

Vital capacity, in cubic centimeters, as obtained on Pike's Peak

SUBJECT	R. A.	H. G.	L. R.	E. S. 1	D. S.	Q. C.	W. R.	E. S. 2
Colo. Springs								
Smallest	5,880	3,920	5,300	4,250	3,800	4,510	4,200	4,480
Largest	6,020	3,950	5,490	4,450	3,970	4,600	4,310	4,510
Pike's Peak								
Day 1	5,100	3,550	5,050	4,020	3,600	4,200	3,990	4,160
2	5,480	3,530	4,990	4,000	3,530	4,310	4,100	4,260
3	5,430	3,700	5,150	4,100	3,600	4,480	4,140	4,370
4	5,600	3,710	4,950	4,200	3,650	4,510	4,170	4,440
5	5,550	3,675	5,230	4,125	3,710	4,500	4,200	4,400
6			5,200	4,100	3,700	4,380	—	—
7			5,230	4,025	3,600	4,360	4,070	—
8			5,220	4,275	3,790	4,530	4,300	4,440
9			5,210	—	3,690	4,515	4,190	4,440
10			—	—	—			
11			5,190	4,275	—			
11			—	—	—			
12			5,190	4,275	—			
13			5,300	4,250	3,680			

As a rule the maximum decrease was obtained during the first day of residence on the mountain, but in several of the men it occurred on the second. The ascent of the mountain was ordinarily made by cog-railway train, but four of our subjects climbed the mountain afoot. It was the men of the latter group who showed the largest reduction in the vital capacity, 10.6 to 15.3 per cent (see R. A. and H. G., table 3).

In all of our subjects the vital capacity sooner or later showed a marked tendency to return to normal. This was usually in evidence by the second day of residence, or at least by the third day. In two of the men, who walked up the mountain, the beginning of the recovery was delayed five

days; while in one, L. H., who ascended passively on the railway train, there was an equal delay in two expeditions. Among the men who remained from 9 to 13 days on the mountain the return to normal in vital capacity was almost, but never quite, complete. The data in table 3 show that the return for L. H., E. S., G. C. and W. R. was sufficient to bring it within the limits obtained at the low altitude; but that W. R. alone, while on Pike's Peak, had a vital capacity equal to his maximum determination at Colorado Springs, the low altitude.

In three journeys to the summit the vital capacity of E. S. was reduced by 7.1, 9.3, and 10.1 per cent respectively; and during each of them the recovery was approximately the same. In two trips D. S. gave reductions of 11.7 and 11.1 per cent and L. H. of 6.7 and 8 per cent respectively. These observations indicate that the decrease in barometric pressure reduces the vital capacity of the lungs to quite the same degree with every ascent of the mountain.

Observations on one man, who resided on the summit of Pike's Peak from early in May to November, indicate that with complete acclimatization the vital capacity at the high altitude comes to be the same as at low altitudes. Repeated tests were made on this man while he was on the summit of the mountain during a period of five days in October, and showed his vital capacity to range between 3950 and 4200 cc. The following winter, while he was in Colorado Springs, the determinations gave a range between 3970 and 4220 cc.

The effect of oxygen while on Pike's Peak. On two days, about the middle of a 14 day sojourn on the mountain, oxygen was administered for a period of 15 minutes to three of our subjects. In each of the six trials the breathing of oxygen resulted in some increase in the vital capacity, but in no instance did it restore the capacity to the maximum of Colorado Springs. The best result obtained for each man with the use of oxygen was as follows: L.H. was raised from 5120 to 5300 cc.; E.S., from 4000 to 4250 cc.; and D.S., from 3620 to 3800 cc. In view of the fact that the funnel method of administering oxygen was employed, it is questionable whether we succeeded in raising the alveolar oxygen pressure in the lungs to that found at the low altitude. Hence we may conclude that the reduction in the vital capacity of the lungs, which is experienced at high altitudes in the mountains, is a result of anoxemia.

The effect of atmospheric cold. Since Fuchs made the suggestion that the decrease in vital capacity at high altitudes is due to an increase in muscle-tone caused by low atmospheric temperature, we decided to test out this point. On two different days two of the men remained out of doors, quite inactive, for upwards of an hour. During this time the vital capacity was repeatedly determined by passing the pipe of the spirometer to them through a crack in a door. In no instance was the vital capacity of these

men lessened by their exposure to temperatures of 16° and 18°F. The other data we have reported were obtained in a room at temperatures ranging from 67° to 72°F.

The cause of the decrease in vital capacity. Our observations in the low pressure chamber and on Pike's Peak seem to refute some of the theories offered to explain the reduction in vital capacity. They tend to eliminate the theories which attribute the change to an expansion of intestinal gases or to a tonic effect of low temperatures. They tend to cast doubt on the theory that attributes the reduction to fatigue of the respiratory muscles. It is true that men who walked up Pike's Peak, on the whole, experienced the greatest reduction; but the differences were not proportionate to the fatigue. One of those men, H.G. table 3, was extremely exhausted and prostrated, yet he had a reduction of only 10.6 per cent; while R.A., who arrived feeling very well, showed a decrease of 15.3 per cent in his vital capacity. R. A., 15 minutes after arriving on summit, had a vital capacity of 5600 cc.; two hours later, it had fallen to 5450 cc.; and five hours after the ascent, it was only 5100 cc., which would seem to eliminate fatigue as a cause.

The work of Drinker, Peabody and Blumgart (1922) on the effect of pulmonary congestion on the vital capacity of the lungs in heart disease shows that an engorgement of the pulmonary blood vessels may take up alveolar space which air could occupy under normal conditions. In 1903 Bartlett presented experiments that showed that the pulmonary vessels, when exposed to diminished air pressure, dilate and thereby produce congestion in the lesser circulation. This he attributed to an equalization of the atmospheric and the intrathoracic pressures. Schubert (1930) similarly believes that in rarefied air the elastic force of the alveoli has less to oppose, consequently the alveolar walls relax and their lumen becomes smaller. Along with this the capillaries enlarge and passive congestion follows. It seems unlikely, in view of the recovery in vital capacity that we obtained during oxygen inhalation, that Bartlett's and Schubert's explanations can be applied to our problem. Grollman (1930) found that the heart output per minute in two subjects while on Pike's Peak increased steadily for four or five days, reaching a maximum of about 40 per cent above its sea-level value and then gradually declined to its normal level. This is the inverse of the series of changes some of our subjects experienced in the vital capacity of their lungs. Conceivably reserve capillary paths may be brought into requisition during the early stages of acclimatization. Vannotti (1931) was able to identify particular capillary loops in the skin and in these definite dilatation was observed at high altitudes. It may be that the anoxemia experienced under low barometric pressure causes a relaxation or loss of tonus of the pulmonary capillaries, and that this in turn is followed by capillary engorgement; or that sluggish and empty capil-

lary areas may be brought into active circulation. The beneficial effect we obtained by administering oxygen would very likely be on the capillary endothelium which was thus enabled to return to or toward its natural tonus.

SUMMARY

A decrease in the vital capacity of the lungs was obtained by subjecting men to a reduced barometric pressure in a low pressure chamber. Individual differences occurred; no one experienced the change at less than a simulated altitude of 10,000 feet; while in the majority of cases this occurred at simulated altitudes of from 12,000 to 15,000 feet. The use of an extra supply of oxygen prevented the effect in part in some men and wholly in others.

By measuring the circumference of the abdomen, it was shown that the decrease in vital capacity is not the result of the expansion of intestinal gases.

On Pike's Peak, altitude 14,110 feet, the vital capacity was decreased during the first day or two by from 6.7 to 15.3 per cent in nine men. Later all men showed at least a partial return to normal. With acclimatization the recovery was complete. During the period of reduced vital capacity the administration of oxygen caused a partial recovery.

A low atmospheric temperature was shown not to be the cause of the decrease.

It is suggested that the reduction in vital capacity is due to an engorgement of pulmonary blood vessels, that reserve capillary paths are brought into requisition, and that possibly there is a relaxation of pulmonary capillaries as a result of anoxemia. The beneficial effect obtained by the administration of oxygen may be found to be due to restored capillary tonus.

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SOME PHASES OF LIVER AND GALL-BLADDER FUNCTION

Experiments on Rabbits with Certain Organic Compounds, Particularly Dyes

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A substance that is excreted into the bile, that will accumulate in the gall bladder, that is opaque to Roentgen rays and that possesses bactericidal properties would probably be of value in the diagnosis and treatment of certain diseases of the gall bladder. The procedure used in our former experiments provides a simple direct method for determining whether or not a chemical compound is excreted into the bile and if it accumulates in the gall bladder (in animals).

In this preliminary investigation we have studied four dyes, namely, tetraiodophenolphthalein and the closely related flourescein derivatives: eosin, erythrosin and rose bengal, as well as sodium salicylate and the sodium salt of diiodosalicylic acid.[1]

Experimental procedures. *Surgical procedure.* The method was identical with that previously described (1).

Mode of administration. The compounds studied were either injected intravenously or administered orally (by stomach tube).

Analytical methods. The fluorescein derivatives, *eosin*, *erythrosin* and *rose bengal*, are so intensely colored that a special chemical method is not required for their determination. The excreted bile and the bile in the gall bladder are colored intensely red as soon as the excretion of the dye has begun. It usually suffices, therefore, to dilute the bile accurately to some convenient concentration and compare this solution, in a colorimeter, with a standard solution of the dye. The inherent color of the bile is a negligible factor until the concentration of the dye has become so minute (1:10,000) that it too becomes negligible.

Salicylic acid was determined as follows: Bile or urine—10 cc.—is mixed with 90 cc. of a saturated solution of sodium bicarbonate. The

[1] A brief summary of the results of some of these experiments was read (by title) at the Forty-fifth Session of the American Association of Anatomists (4) and at the XIIIth International Physiological Congress (6).

resulting liquid is extracted three times with ether using 200 cc. for each extraction. The ether extract is discarded. Neutralize the alkaline solution with 7N sulfuric acid (congo red paper) and add 2 cc. of sulfuric acid in excess. The acid liquid is extracted three times with ether using 200 cc. for each extraction. The combined ether extracts, which contain the salicylic acid, are evaporated in a glass dish at room temperature by means of a current of dry air. A saturated solution of sodium bicarbonate —1.0 to 1.5 cc.—is added to the residue in the glass dish. Transfer, with water, to a 20 cc. volumetric flask and dilute to the mark. The salicylic acid is determined in 10 cc. of this liquid by the Folin and Ciocalteu modification of the Millon reaction (5). The color is compared with that produced by 5 mgm. of salicylic acid in 10 cc. of a solution of an alkalinity equivalent to that of the test solution.

Diiodosalicylic acid and *tetra iodophenolphthalein* were not determined directly. Bile and urine were analyzed for iodine (method below) and the amounts of the diiodosalicylic acid or of the tetra iodophenolphthalein determined by calculation.

Method for determining iodine in bile or urine. The bile or urine (1 cc.) is measured into a silver crucible and evaporated to dryness on the steam bath. Place 20 grams of potassium hydroxide in another silver crucible and heat until all the water is driven off. Pour the molten KOH into the crucible containing the dried bile or urine and heat carefully until the melt is a clear, straw colored liquid. The fusion usually takes 3 to 4 minutes. Allow the material to cool and transfer quantitatively, with distilled water, to a 500 cc. Erlenmeyer flask. The transfer should involve the use of about 125 cc. of water. Acidify with 40 cc. of 85 per cent phosphoric acid, add 50 cc. of saturated chlorine water, and boil. Considerable water must be vaporized before the liquid is free from Cl_2. Additional water may have to be added. The absence of Cl_2 can be determined by the usual starch KI method. *Cool,* add a pinch of KI and starch solution (about 1 cc.), and titrate immediately with 0.02 N sodium thiosulfate.

Experiments with eosin (*a*), *erythrosin* (*b*), *and rose bengal* (*c*). It is mentioned by Graham and his collaborators that the above halogenated derivatives of fluorescein produce cholecystograms (2). Staining of the tissues, however, precluded their clinical use. The following are data on the mechanism of their excretion.

a. Eosin (tetrabromfluorescein) injected intravenously in doses of 20 mgm. (i.e., 2 cc. of a 1 per cent solution) per kgm. of body weight, appeared in the bile in three to five minutes; it reached the highest concentration in the bile collected from the ductus choledochus within 24 to 42 minutes. This maximum concentration—1:51 to 1:122—was approximately maintained for from 28 to 53 minutes. Values ranging between 1:2,900 and 1:14,000 were obtained 6 hours after the injection. The bile removed

from the gall bladder at the same time, i.e., 6 hours after the injection, contained eosin in a higher concentration than the last sample of bile obtained from the ductus choledochus (table 1). In each animal the concentration of eosin in the bile removed from the gall bladder, however, was always less than the highest concentration reached in the bile collected from the ductus choledochus.

b. Erythrosin (tetraiodofluorescein) injected intravenously in doses of 20 mgm. (i.e., 2 cc. of a 1 per cent solution) per kgm. of body weight, appeared in the bile within 3 to 4 minutes; it reached the highest concentration in the bile collected from the ductus choledochus within 23 to 33 minutes. This maximum concentration—1:80 to 1:200— was approximately maintained for from 14 to 67 minutes. Values ranging between 1:900 and 1:20,000 were obtained 6 hours after the injection. The bile removed from the gall bladder at the same time, i.e., 6 hours after the injection, contained from 10 to 7 hundred times as much erythrosin as that contained in the last sample of bile obtained from the ductus choledochus (table 1). The concentration of erythrosin in the bile removed from the

TABLE 1

Summary of data on the excretion of eosin, erythrosin, and rose bengal by the biliary system of the rabbit following their intravenous administration in doses of 20 mgm. (i.e., 2 cc. of a 1 per cent solution) per kgm. of body weight

RABBIT	FIRST APPEARANCE OF DYE IN THE BILE	HIGHEST CONCENTRATION OF DYE IN THE BILE FROM D. CHOLEDOCHUS			DURATION OF EXPERIMENT	AVERAGE FLOW OF BILE PER HOUR	CONCENTRATION OF DYE IN BILE AT CLOSE OF EXPERIMENT		
		Reached within	Dilution	Approximately maintained for			From D. choledochus	From gall bladder	Ratio of concentration approximate
				Eosin					
	minutes	*minutes*		*minutes*	*hours*	*cc.*			
I-1	4	24	1:51	28	6	6.3	1:4,700	1:90	1:52
I-3	3	29	1:100	53	6	4.3	1:2,900	1:2,000	1:1.4
I-2	5	42	1:122	42	6	3.7	1:11,600	1:3,060	1:3.7
				Erythrosin					
I-3	4	25	1:80	17	6	7.5	1:20,000	1:28	1:714
I-2	3	23	1:100	14	6	11.4	1:11,500	1:720	1:15.8
I-1	4	33	1:200	67	6	2.7	1:900	1:90	1:10
				Rose bengal					
I-3	5	55	1:142	75	6	3.5	1:1,180	1:750	1:1.5
I-1	7	65	1:144	15	3	5	1:960	—	—
I-2	6	32	1:192	35	2	4.5	1:340	1:252	1:1.3

gall bladder at the end of the sixth hour following the injection was higher than the highest concentration that it reached in the bile collected from the ductus choledochus. This was true in two out of three animals.

In one rabbit 40 mgm. (i.e., 2 cc. of a 2 per cent solution) of erythrosin per kgm. of body weight were injected intravenously. The results closely resembled those obtained by the injection of half this amount, i.e., the dye appeared in the bile about 3 minutes following the injection; it reached the highest concentration in the bile collected from the ductus choledochus within 30 minutes; this maximum concentration—1:63—was approximately maintained for about 40 minutes; 3 hours following the injection the concentration of erythrosin in the bile collected from the ductus choledochus was 1:2000, in that removed from the gall bladder 1:45.

Erythrosin could not be detected in the bile collected from the ductus choledochus or in that removed from the gall bladder 24 hours following the administration to one rabbit intravenously and to another by stomach tube of 20 mgm. (i.e., 2 cc. of a 1 per cent solution) of erythrosin per kgm. of body weight.

c. *Rose bengal* (tetraiodotetrachlorfluorescein) injected intravenously in doses of 20 mgm. (i.e., 2 cc. of a 1 per cent solution) per kgm. of body weight appeared in the bile in 5 to 7 minutes; it reached the highest concentration in the bile collected from the ductus choledochus in 32 to 65 minutes. This maximum concentration—1:42 to 1:192—was approximately maintained for from 15 to 75 minutes. The values obtained in 3 animals, 2, 3 and 6 hours following the injection were 1:340, 1:960, and 1:1,180 respectively. The bile removed from the gall bladder at the same time contained rose bengal in a concentration of 1:252 two hours, and 1:750 six hours following the injection (table 1). Thus the concentration of rose bengal 2 and 6 hours following the administration was higher in the bile removed from the gall bladder than in the sample of bile collected from the ductus choledochus. This concentration, however, was less than the highest concentration reached in the bile collected from the ductus choledochus.

Rose bengal cannot be determined in the bile collected from the ductus choledochus 24 hours after the intravenous administration of the dye. Rose bengal is, however, still present in the gall bladder at the end of this time interval. The gall bladder bile of 3 rabbits that had received 40 mgm. of rose bengal per kgm. of body weight contained the dye in concentrations of 1:46, 1:73 and 1:118 respectively; the gall bladder bile of 3 rabbits that received 60 mgm. of rose bengal per kgm. of body weight contained the dye in concentrations of 1:60, 1:111 and 1:230 respectively, 24 hours after injection.

Rose bengal cannot be determined in gall-bladder bile 48 hours after the dye has been injected.

Excretion of the dyes into the bile continued for several hours even when the dyes were administered intravenously. This would suggest that at least a portion of the dye remained in the blood stream. The presence of the dyes could not, however, be determined by a direct examination of the blood. Better results were obtained by an indirect method. The procedure adopted was that of removing the aqueous humor twice in succession from one eye, using the other eye as a control. These experiments were carried out upon the suggestion and with the coöperation of Dr. Peter C. Kronfeld of the Department of Ophthalmology, University of Chicago. None of these dyes (eosin, erythrosin, rose bengal) appeared spontaneously in the aqueous humor removed one and two hours following the intravenous administration of the dyes in doses of 20 mgm. (i.e., 2 cc. of a 1 per cent solution) per kgm. of body weight. A second sample of the aqueous humor, on the other hand, removed 15 minutes after the first, invariably contained the dye (3).

Experiments with sodium salicylate (*a*), *and sodium salt of diiodosalicylic acid* (*b*). In view of the differences of opinion regarding the action of salicylates on the biliary system as cholagogues and as bactericides, it seemed desirable to investigate the excretion of the above two representatives of the group.

a. *Sodium salicylate* was given intravenously in doses of 40 mgm. (i.e., 4 cc. of 1 per cent solution) per kgm. of body weight, and the bile collected in the usual manner in 1 cc. samples for a period of 3 hours in one rabbit and 4 in another. The flow of bile averaged 5 and 9 cc. per hour. Neither these samples, nor the bile removed from the gall bladder at the end of the experiment, contained salicylates in determinable quantities (4).

b. *Sodium salt of diiodosalicylic acid* was given intravenously in doses of 40 gm. (i.e., 4 cc. of a 1 per cent solution) per kgm. of body weight, and the bile collected in the usual manner in 1 cc. samples for a period of 2 hours in one rabbit and 3 in another. The flow of bile averaged about 5.5 and 10 cc. per hour. Iodine, and therefore diiodosalicylic acid, was not present in determinable quantities in any of the samples of bile collected from the ductus choledochus or in that removed from the gall bladder at the end of the experiment.

Experiments with sodium salt of tetraiodophenolphthalein. With the object of securing quantitative data on the mechanism of the excretion with this dye, which is most extensively used for visualizing the gall bladder, three sets of experiments were performed. In the first series, *a*, sodium salt of tetraiodophenolphthalein (Iodeikon—Mallinckrodt) was given intravenously in doses of 20, 40 and 60 mgm. (i.e., 1, 2 and 3 cc. of a 2 per cent solution) per kgm. of body weight, and the bile collected in the usual manner in 1 cc. samples for a period of 6 hours. In the second series, *b*, iodeikon was injected intravenously in doses of 20 mgm. (i.e., 1 cc. of a 2

per cent solution) per kgm. of body weight and the operation performed at certain stipulated intervals between the 24th and 96th hours following the administration. In the third series, *c*, the same amount of iodeikon,

TABLE 2

Summary of data on the excretion of iodeikon by the biliary system of the rabbit following intravenous administration of 20, 40, and 60 mgm. (i.e., 1, 2, and 3 cc. of a 2 per cent solution) respectively per kgm. of body weight

RABBIT	FIRST APPEARANCE OF DYE IN THE BILE	HIGHEST CONCENTRATION OF DYE IN THE BILE FROM D. CHOLEDOCHUS			DURATION OF EXPERIMENT	AVERAGE FLOW OF BILE PER HOUR	CONCENTRATION OF DYE IN BILE AT CLOSE OF EXPERIMENT		
		Reached within	Dilution	Approximately maintained for			From D. choledochus	From gall bladder	Ratio of concentration approximate
	minutes	*minutes*		*minutes*	*hours*	*cc.*			
I-10	20	39	1:136	30	6	2	1:747	1:927	1.2:1
I-8	7	40	1:209	40	6	2.2	1:1,215	1:1,195	1:1
I-7	8	35	1:133	60	6	6.6	1:5,160	—	—
I-6	15	60	1:143	30	6	2.2	1:554	1:452	1:1.2
I-9	5	55	1:31	60	6	3.1	1:92	1:18	1:5.1
I-11	16	110	1:78	75	6	2	1:420	—	—

TABLE 3

Concentration of the dye in the bile collected from the cannulated ductus choledochus and in the bile removed from the gall bladder of the rabbit 24, 48, 72, and 96 hours following intravenous administration of 20 mgm. (i.e., 1 cc. of a 2 per cent solution) of iodeikon per kgm. of body weight

RABBIT	D. CHOLEDOCHUS	GALL BLADDER	URINE	RABBIT	D. CHOLEDOCHUS	GALL BLADDER	URINE
24-hour group				72-hour group			
I-1	1:17,000	1:1,615	None	I-9	None	1:2,375	None
I-2	1:2,830	1:3,050	None	I-8	None	1:5,370	—
I-4	1:17,000	1:5,680	None	I-7	None	1:7,880	None
48-hour group				96-hour group			
I-5	None	1:5,100	None	I-10	None	1:5,100	None
I-3	1:12,800	1:6,010	None	I-12	None	1:11,900	—
I-6	None	1:7,750	None	I-11	None	None	—

(i.e., 1 cc. of a 2 per cent solution) per kgm. of body weight, was given by stomach tube and the operation performed 24, 48, 72 and 96 hours later.

The results obtained are charted in tables 2, 3 and 4, and may be sum-

marized briefly as follows: *a.* Iodeikon injected intravenously in doses of 20, 40 and 60 mgm. (i.e., 1, 2 and 3 cc. of a 2 per cent solution) per kgm. of body weight, led to the appearance of iodine in the bile in 5 to 20 minutes; it reached the highest concentration in the bile collected from the ductus choledochus in 35 to 110 minutes. The maximum concentration of the dye (as calculated from the iodine content)—1:31 to 1:209—was approximately maintained for from 30 to 75 minutes. Values ranging between 1:92 and 1:5,160 were obtained 6 hours after the injection. The bile removed from the gall bladder at the same time, i.e., 6 hours after the injection, contained about 1 to 5 times as much iodeikon as that contained in the last sample of bile obtained from the ductus choledochus (table 2). *b.* The dye was present in the bile collected from the ductus

TABLE 4

Concentration of the dye in the bile collected from the cannulated ductus choledochus and in the bile removed from the gall bladder of the rabbit 24, 48, 72, and 96 hours following oral administration of 20 mgm. (i.e., 1 cc. of a 2 per cent solution) of iodeikon per kgm. of body weight

RABBIT	D. CHOLEDOCHUS	GALL BLADDER	URINE	RABBIT	D. CHOLEDOCHUS	GALL BLADDER	URINE
24-hour group				72-hour group			
F-5	1:17,000	1:8,170	None	F-4	None	1:1,700	None
F-7	None	1:9,000	None	F-8	None	1:17,000	—
R-6	None	1:10,200	None	F-9	None	None	—
48-hour group				96-hour group			
F-1	1:17,000	1:1,510	None	F-10	None	None	None
F-2	1:17,000	1:2,550	None	F-11	None	None	None
F-3	None	1:6,150	None	F-12	None	—	None

choledochus in concentrations of between 1:2,830 and 1:17,000, and in the gall bladder in concentrations of 1:1,615 to 1:7,750, 24 and 48 hours following intravenous administration of iodeikon in doses of 20 mgm. per kgm. of body weight. Iodeikon could not be detected in the bile collected from the ductus choledochus 72 and 96 hours following administration; the gall bladder on the other hand contained iodeikon in concentrations from 1:2,375 to 1:11,900 (table 3). *c.* The dye was detected in the bile collected from the ductus choledochus in 3 of the 6 rabbits in a concentration of 1:17,000, and in the gall bladder in concentrations that varied between 1:1,510 and 1:10,200, 24 and 48 hours following oral administration of iodeikon in doses of 20 mgm. per kgm. of body weight. Iodeikon was not found in the bile collected from the ductus choledochus 72 and 96 hours following the administration; but the gall bladder con-

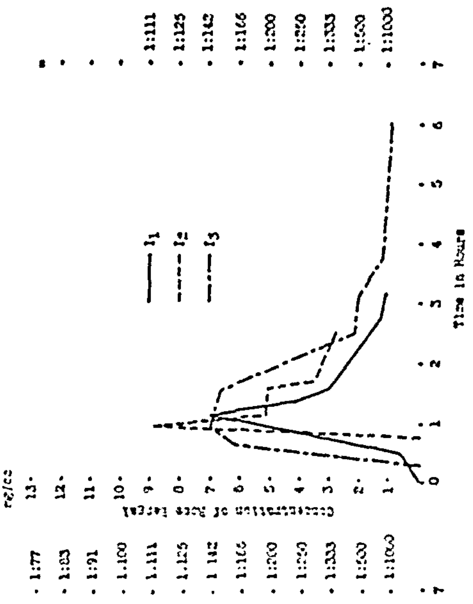

Fig. 3

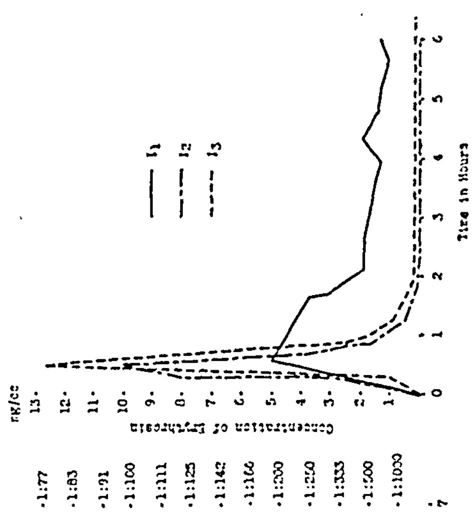

Fig. 2

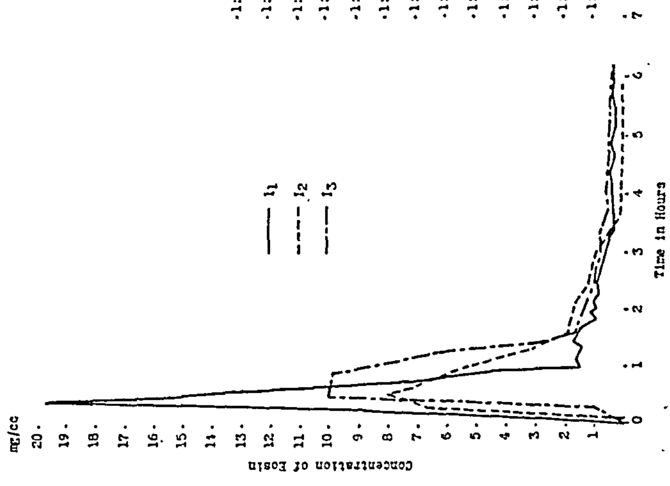

Fig. 1

tained iodeikon in 2 of the 6 rabbits in concentrations of 1:1,700 and 1:17,000.

COMMENT. All three of the halogenated derivatives of fluorescein studied appeared in the bile within a few minutes following their intravenous administration in doses of 20 mgm. per kgm. of body weight and invariably remained in the bloodstream for at least two hours. The curve indicating the dye content of the bile collected from the ductus choledochus was a regular one: a sharp rise was followed by a sudden decline (figs. 1, 2 and 3). In all instances the concentration of the dye was higher in the bile from the gall bladder than in the bile collected from the ductus choledochus 6 hours after the intravenous administration of eosin, erythrosin or rose bengal in spite of the fact that the gall bladder was never empty to begin with and that during this entire period of the experiment bile was passing through the cannulated ductus choledochus at a rate of 2.7 to 11.4 cc. per hour. Of these three dyes only erythrosin showed a higher concentration in the bile from the gall bladder than the maximum concentration ever reached in the bile collected from the ductus choledochus (table 1). Twenty-four hours following intravenous administration of rose bengal in doses of 40 and 60 mgm. per kgm. of body weight, the dye appeared in the bile removed from the gall bladder in concentrations between 1:46 and 1:230, and none in the bile collected from the ductus choledochus.

The experiments with sodium salicylate and the sodium salt of diiodosalicylic acid brought out the remarkable fact that these substances do not appear in the bile in determinable quantities after their intravenous administration in doses of 40 mgm. per kgm. of body weight.

SUMMARY

Eosin, erythrosin, or rose bengal injected intravenously into rabbits, in doses of 20 mgm. per kgm. of body weight, appeared in the bile within a few minutes. The bile collected from the ductus choledochus showed a sharp rise to the highest concentration—1:51 to 1:200—followed by a sudden decline. In all instances the concentration of the dye in the bile collected from the ductus choledochus 6 hours after the injection was less than in the bile simultaneously removed from the gall bladder, in spite of the fact that during the entire period of the experiment bile was passing through the cannulated ductus choledochus. Rose bengal was present in the bile removed from the gall bladder in concentrations between 1:46 and 1:230 when none was present in the bile collected from the ductus choledochus 24 hours following intravenous administration of the dye in doses of 40 and 60 mgm. per kgm. of body weight.

Sodium salicylate or the sodium salt of diiodosalicylic acid injected intravenously in doses of 40 mgm. per kgm. of body weight did not appear in the bile in determinable quantities.

The excretion of iodine into the bile continues for 48 hours after the sodium salt of tetraiodophenolphthalein is given either intravenously or by mouth. It may sometimes be found in the gall bladder for as long as 96 hours.

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THE ACTION OF CERTAIN DRUGS ON THE NICTITATING MEMBRANE

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During the study of the innervation of the nictitating membrane (n.m.) of the cat (Rosenblueth and Bard, 1932) one of the methods first used was that of examining the action of sympatho- and parasympathomimetic drugs on the organ. The results did not help to solve the problem then in view, in fact they appeared confusing and paradoxical when confronted with the data supplied by other methods. The action of the parasympathomimetic drugs on the n.m. is an exception to their usual effects. It was considered interesting to investigate further this paradoxical behavior.

Cats were used. The movements of the n.m. were recorded by the method described by Rosenblueth and Cannon (1932). The influence of previous denervation of the n.m. (right superior cervical ganglionectomy performed at least six days before) was studied, using the other membrane, acutely denervated, as a control. The contractions of both membranes were sometimes simultaneously recorded. Two levers of the same length and weight were adjusted to produce the same amplification and attached to corresponding points in the membranes. The head was fixed upright in a horizontal position and the levers were attached symmetrically.

Although the presence of the adrenals did not seem to affect the responses, these glands were removed or ligated *en masse* in all instances, to ensure a more steady preparation.

Dial anesthesia (between 0.6 cc. and 0.8 cc., intraperitoneally) was used throughout.

The drugs were injected into the femoral vein, through a glass canula. The rate was practically uniform (1 cc. per 5 seconds). The volume of each injection was usually 1 cc.

1. *Adrenalin.* The retracting action of adrenin on the n.m. has been long known. This action is sensitized (i.e., increased) by previous denervation and by cocaine (Rosenblueth and Cannon, loc. cit.). The sensitizing action of cocaine is more marked on the acutely denervated membrane.

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2. *Acetylcholine* causes a retraction of the n.m. (see figs. 1 and 2). This action is sensitized by previous denervation (see fig. 1) and by cocaine (see fig. 2). Atropine abolishes the effect of acetylcholine. The sensitization by cocaine is not as marked as the sensitization by this drug to adrenin.

3. *Pilocarpine* has the same action as acetylcholine. The responses are usually longer and irregular (i.e., the curve is not smooth). The action of pilocarpine had been previously reported by Dale and Laidlaw (1912).

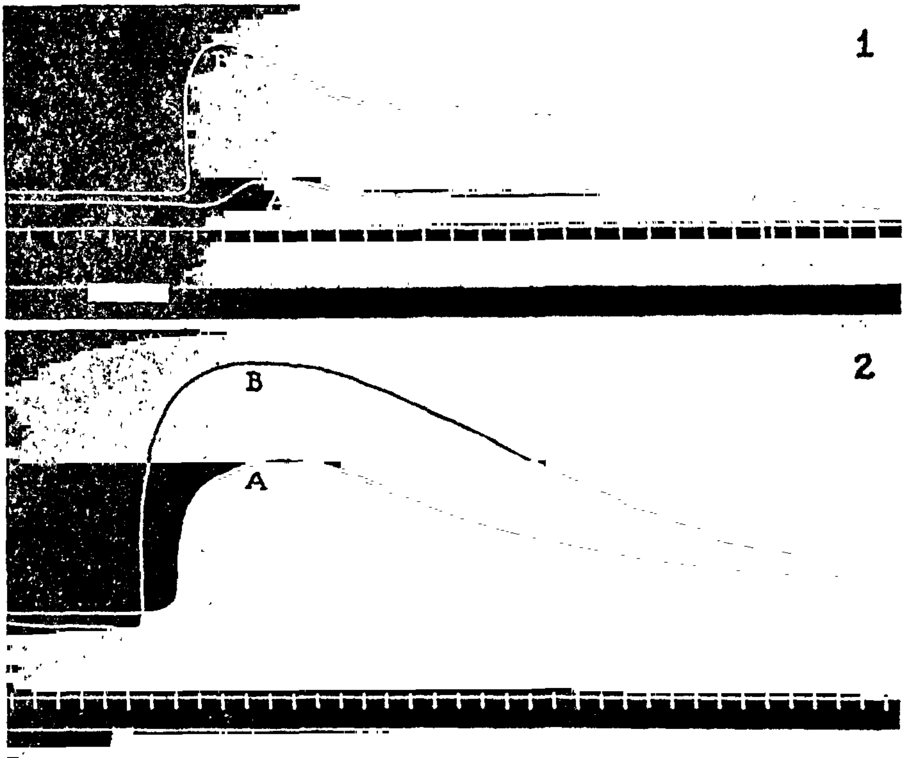

Fig. 1. Simultaneous record of the contraction of both nictitating membranes on injection of 0.5 cc. acetylcholine bromide, 1:100,000. Right n.m., *B*, denervated 6 days previously; left n.m., *A*, denervated acutely. Weight of the cat: 4.3 kgm. Dial: 0.7 cc. intraperitoneally.

Fig. 2. Contraction of the acutely denervated nictitating membrane on injection of 1 cc. acetylcholine bromide, 1:50,000, *A*, before, and *B*, after 23 mgm. cocaine. Weight of the cat: 3.1 kgm. Dial: 0.7 cc. per kilo intraperitoneally.

4. *Physostigmine* also has effects similar to those of acetylcholine. The responses are, however, usually comparatively small, and the sensitization less marked, especially when cocaine is used as a sensitizer. The response is likewise abolished by atropine.

5. *Atropine per se* has no action on the n.m.

6. *Histamine* contracts the n.m. The effects are increased by previous

denervation and by cocaine. Atropine in doses sufficient to antagonize the action of pilocarpine, acetylcholine and physostigmine (2 to 3 mgm. per kilo) does not abolish the response, although the effect may decrease slightly.

7. *Ergotoxine* (up to 9 mgm. per kilo) causes a permanent contraction of the n.m. The contraction is about the same in the acutely and the chronically denervated membranes. If adrenalin is then injected there appears a transitory slight relaxation. The same effects were observed by Dale (1906).

DISCUSSION. Since it is well established that the n.m. possesses a retractor smooth-muscle mechanism controlled by the sympathetic, the action of adrenin need not be commented upon.

The action of parasympathomimetic drugs may be explained in one of the following hypotheses:

a. We may be dealing with amphoteric drugs with marked predominance of action on the parasympathetic endings; when these are absent, the effect on the sympathetic endings would assert itself.

b. The sympathetic receptors of the n.m. may be exceptional in that they may be stimulated both by sympathomimetic and by parasympathomimetic drugs.

c. There may exist in different smooth muscle cells in the organism parasympathetic receptor mechanisms not connected with any nerves. This last hypothesis was suggested by Dale and Laidlaw (loc. cit.) and adopted by Hunt (1918) to explain the vasodilating effect of acetylcholine. This explanation seems improbable, however, for in the case of the n.m. the parasympathetic receptor would be acting in the same direction as does the sympathetic innervation of the muscle.

The first two hypotheses constitute really a single assumption: that the receptor mechanism connected normally with the sympathetic is susceptible of being acted upon by other substances than adrenin, including the parasympathomimetic drugs. That this view is probably correct is indicated by the fact that previous sympathetic denervation and cocaine, which typically sensitize to adrenin, also sensitize to the parasympathomimetic drugs, pilocarpine and acetylcholine. Sympathetic denervation should have no effect on parasympathetic receptors. As for cocaine, three experiments showed that it did not sensitize the action of acetylcholine and pilocarpine on the blood pressure.

The action of ergotoxine may be explained as a direct stimulation of the smooth muscle, similar to that exerted on the uterus.

The action of histamine appears similar to that of the parasympathomimetic drugs, being also affected by the sensitizers.

Physostigmine appears here as a parasympathomimetic drug, its effects being abolished by atropine.

SUMMARY

Acetylcholine, pilocarpine and physostigmine produce contraction of the smooth muscle in the nictitating membrane of the cat. This action is increased by previous denervation (see fig. 1) and by cocaine (see fig. 2), and is abolished by atropine.

Histamine has a similar effect, but atropine does not interfere with its action.

Ergotoxine causes a permanent contraction of the nictitating membrane and reverses the action of adrenin.

It is pointed out that the parasympathomimetic drugs act probably on the same receptor mechanism on which adrenin acts.

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FURTHER STUDIES IN MUSCLE FATIGUE AND PERMEABILITY[1]

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In previous papers (Gellhorn, 1930–1931) it has been found that fatigue in striated frog's muscles can be delayed considerably by immersing them in a Ringer's solution with a calcium chloride concentration higher than normal. The experiments seemed to indicate that fatigue is accompanied by an increase in permeability and can be remedied to a certain extent by an excess of calcium ions. Whereas, in the investigations cited above the fatigue was brought about by *direct* stimulation of muscles with condenser discharges, the present paper deals with *indirect* stimulation. The questions to be answered are: 1. Do the experiments support the supposition that *physiological* stimulation also leads to an increase in permeability? 2. If so, do qualitative or quantitative differences exist in the permeability of directly and indirectly stimulated muscles which might be revealed in studies of ion effects in fatigued muscles?

The method was essentially the same as was used in the previous work. The nerve-muscle preparation (*Rana esculenta*) was set up in ordinary fashion and the nerve was stimulated with condenser discharges by means of the apparatus of Scheminsky. To avoid the shock due to the preparation, experiments were begun after the muscle had been immersed for 45 minutes in Ringer's solution. The frequency of stimulation varied in different experiments between 60 and 80 discharges per minute. The calcium chloride content of Ringer's solution (control experiment) was 8.1 M $\times$ 10^{-4}, that of the other solution varied between 32.4 M $\times$ 10^{-4} and 81 M $\times$ 10^{-4}. It was found that 81 M $\times$ 10^{-4} $CaCl_2$ exerted an unfavorable influence on the nerve muscle preparation so that the height of contraction was less than in the control experiment. Therefore smaller

[1] Aided by a grant of the National Research Council and the research fund of the University of Oregon.

concentrations, in most experiments 32.4 M $\times$ 10^{-4} $CaCl_2$, were used. It was found that the time of immersion before the stimulation started had a considerable influence on the efficiency of calcium. If the stimulation began almost immediately after the muscle had been set up, the effect of the B solution[2] was only slightly more favorable than that of the A solution. But if the muscles were immersed for 10 or 15 minutes in their respective solutions before stimulation was applied, the differences were more marked. Therefore all experiments described in this paper were arranged in that manner. In figure 1 a typical example is reproduced. It is evident that in the beginning of the experiments the curves of the two muscles are almost the same, but in the latter part, when fatigue becomes more marked, the B muscle shows a lesser degree of fatigue.

A number of experiments are reproduced in figure 2 in which the height of contraction of the A muscle is taken as 100 throughout the whole experiment and that of the B muscle is calculated in percentages of the first. The result is that up to the fifth or sixth minute, only slight differences if any are observed, but thereafter all the curves rise, indicating that the height of contraction in the B muscle decreases much less than that of the A muscle. At the end of the tenth minute the differences amount to more than 100 per cent in most cases.

The chief difference in comparison with directly stimulated muscles lies in the fact that in the experiments described in this paper, distinct results were obtained only if the solutions had affected the muscles for at least ten minutes, whereas in experiments reported previously characteristic results were also obtained if the stimulation was begun immediately after the muscles had been immersed in their respective solutions. It is probable that this difference is not due to the method of stimulation but to the structure of the muscle since in the previous work the sartorius and in the present studies the gastrocnemius was used. But there seems to be a quantitative difference in both groups. Although the effect of the B solution is considerable as illustrated in figures 1 and 2, it is less than in experiments with direct stimulation. This seems to indicate that direct stimulation leads to a greater increase in permeability of the muscle, which is remedied by calcium chloride, than physiological stimulation through the nerve. The fact, however, that under these circumstances a distinctly favorable effect of calcium chloride (delaying fatigue) is also observed, although the solution does not alter the irritability, shows that the stimulation of the nerve leads to an increase in permeability of the muscle and confirms the conclusion drawn in a recent paper of Gellhorn and Northup. Our findings perfectly agree with experiments of Toda (1930) who also found that calcium delays fatigue.

[2] For the sake of brevity the solution with the higher $CaCl_2$ content is referred to as B, the normal Ringer's solution as A solution.

Further experiments in analogy with our previous work dealt with the question of whether calcium in its effect of delaying fatigue can be replaced by other bivalent cations. The problem was studied in two groups of experiments. In the first different bivalent cations were employed instead of calcium whereas in the second group they were added to a solution containing $CaCl_2$ in a concentration equal to that of Ringer's solution. Experiments were performed with $SrCl_2$, $BaCl_2$, and $MgCl_2$. In the first group the B solution contained either 32.4 M $\times$ 10^{-4} $SrCl_2$ or $BaCl_2$ or $MgCl_2$, $CaCl_2$ being entirely absent. The results are as follows: In no experiment was a favorable effect of those solutions observed, i.e.,

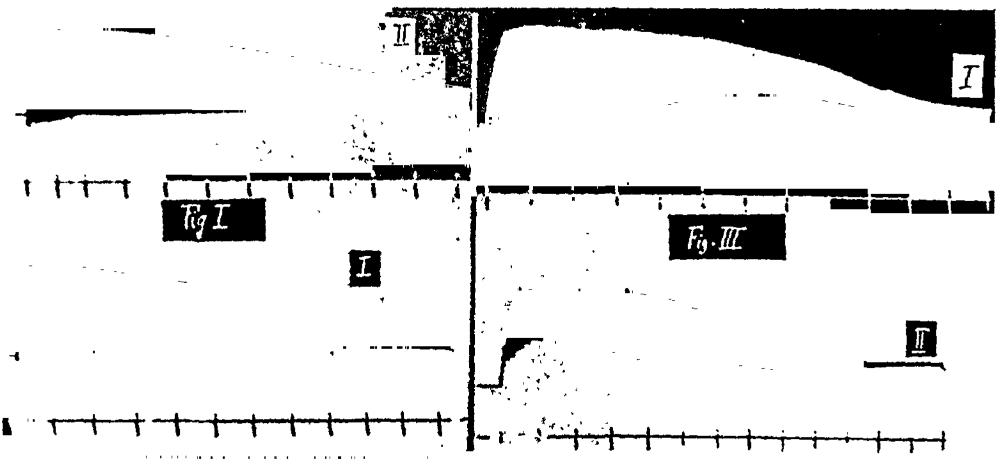

Fig. 1 Fig. 3

Fig. 1. Nerve-muscle preparations. (Rana esculenta.) Maximal condenser discharges 72 times per minute. Indirect stimulation. Muscle I immersed in Ringer's solution with 8.1 M $\times$ 10^{-4}; $CaCl_2$; muscle II in Ringer's with 32.4 M $\times$ 10^{-4} $CaCl_2$. Time in minutes.

Fig. 3. I—Muscle immersed in Ringer's with 8.1 M $\times$ 10^{-4} $CaCl_2$. II—Muscle immersed in Ringer's without $CaCl_2$ + 32.4 M $\times$ 10^{-4} $BaCl_2$.

the fatigue was not delayed in comparison with the course of fatigue observed in Ringer's. In the experiments with $SrCl_2$ and in some with $BaCl_2$ the muscles even showed a slightly greater fatigue than in Ringer's solution. $MgCl_2$ seemed to have no effect whatever on the muscle in the concentrations used in these experiments. A rather interesting observation made in experiments with $BaCl_2$ may be illustrated in figure 3. The curve shows a distinct decrease in the relaxation of the muscle which disappears more or less completely during the course of the experiment. In some observations this contracture effect was less. It reminds one of observations made on sartorius muscle when directly stimulated and immersed in a solution with high $CaCl_2$ concentration (Gellhorn, 1931).

One seems to be dealing here with a phenomenon characteristic of the group of alkali earths. It occurs if by direct or indirect stimulation the permeability of the cell has been sufficiently increased, or if in un-stimulated muscles very high concentrations (isotonic!) of bivalent salts (Guenther, 1905) are used.

Concerning the efficiency of bivalent salts other than calcium, in delaying fatigue, the results are negative, indicating a complete conformity with our previous work on directly stimulated muscles. The calcium effect of delaying fatigue is a specific one. But in the presence of calcium in a concentration of $8.1\ M \times 10^{-4}$ an addition of other bivalent cations

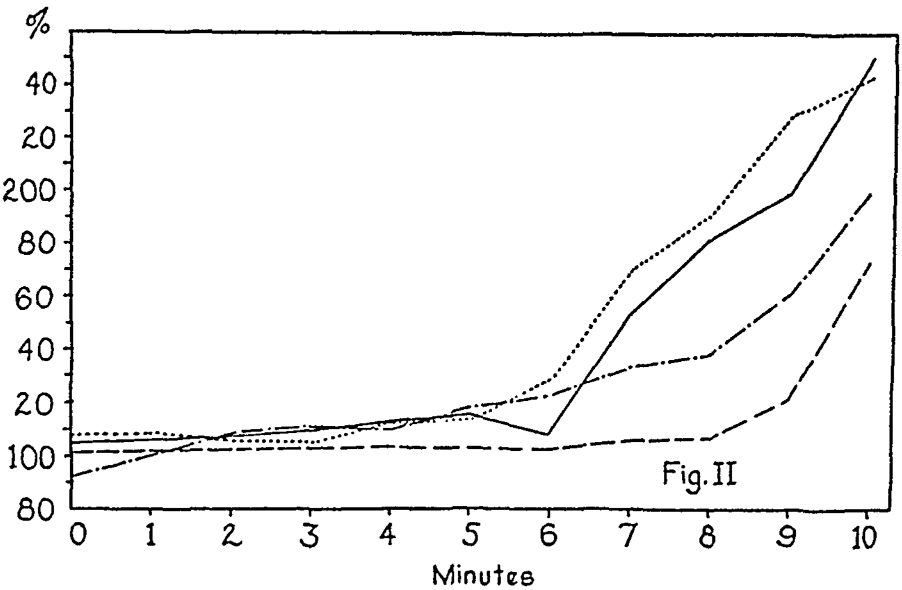

Fig. 2. The height of contraction of muscles immersed in Ringer's with $32.4\ M \times 10^{-4}\ CaCl_2$ expressed as percentage of the height of contraction of the control muscle (Ringer's with $8.1\ M \times 10^{-4}\ CaCl_2$).

is effective although this delay in fatigue is generally less than in a calcium-rich solution.

SUMMARY

Experiments on nerve muscle preparations of *Rana esculenta* with indirect stimulation (condenser discharges) show that in a Ringer solution containing an excess of calcium chloride fatigue is delayed. None of the other alkali earths can replace calcium with the exception that if added to the ordinary calcium content of Ringer's solution, Sr, Mg, and Ba also delay fatigue, in a lesser degree, however, than calcium. $BaCl_2$ causes a contracture of stimulated muscles in very low concentrations. This, as well as the other facts, seems to indicate that the permeability of the muscle is increased by indirect stimulation since the delay of fatigue is obtained in both

directly and indirectly stimulated muscles under the same conditions. The nature of the increase in permeability seems to be the same in both cases if stimulation with super-maximal currents is avoided. The experiments furnish a new illustration of the increase in permeability of muscles under physiological (nerve) stimulation.

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ION EFFECTS ON MUSCULAR FATIGUE AND THEIR INDEPENDENCE OF CHANGES IN THE METABOLISM OF MUSCLES[1]

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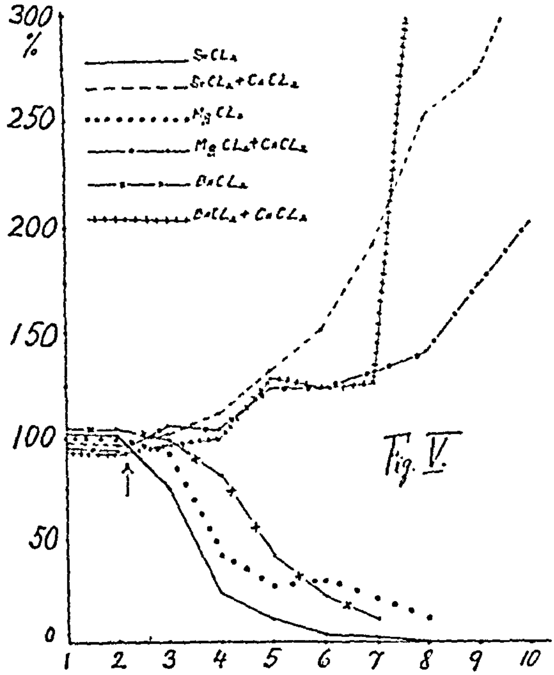
In a preceding paper (Gellhorn, 1932) it has been shown that a close resemblance exists between the ion effects of directly and indirectly stimulated muscles. The conclusion was drawn that the changes in permeability set up by direct stimulation are identical with those observed in experiments with indirect stimulation provided that supramaximal currents are not used.

The present paper has two different purposes: to examine 1, whether the typical ion effects observed in muscles which work on an isotonic lever also hold for isometric contractions; 2, whether the ion effects are altered when the metabolism of the muscle undergoes considerable changes. For the latter purpose the muscles were examined either in a well oxygenated Ringer's solution or in the presence of M/1,000 KCN which suppresses the utilization of oxygen. In a third group of experiments the muscles were previously poisoned with monobromoacetic acid, which according to Lundsgaard (1930) and others suppresses the formation of lactic acid during muscular activity.

METHOD. Nerve muscle preparations of *Rana esculenta* were used during October and December 1931 and were stimulated with interrupted, just maximal, faradic currents (Harvard induction coil with one dry cell in the primary circuit). The tension was recorded by means of an isometric lever (Palmer, London). The nerves of the two nerve muscle preparations of the same frog were placed in series in the secondary circuit so that periods of stimulation and rest were identical. After setting up the muscles in Ringer's solution and placing the nerves over platinum electrodes in a moist chamber above the solution, the preparations were allowed to rest for 10 minutes. Hereafter stimulation began. Two records were taken in Ringer's solution and then one Ringer's solution was replaced by another containing a higher $CaCl_2$ content. In order to make the pro-

[1] Aided by the National Research Council and the Research Fund of the University of Oregon.

cedure as equal as possible the Ringer's solution of the other (control) muscle was exchanged for a fresh Ringer's solution. Time of stimulation varied between 5 and 30 seconds, the intervals between stimulation periods were mostly 5 minutes, in some experiments 2 minutes. Control experiments showed that the method was accurate to an amazing degree. In figure 1 a few records are reproduced which show that the maximal differences between the curves indicating the maximum tension in a num-



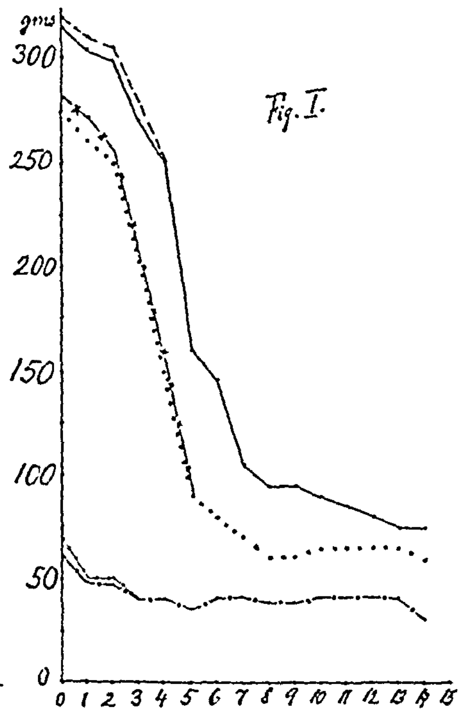


Fig. 1. The two upper curves represent the tension maxima and the lower curves the tension minima of a pair of gastrocnemii which were stimulated indirectly for 20 seconds at intervals of 5 minutes in Ringer's solution. Ordinate: tension; abscissa: number of contractions.

Fig. 5. Explained in the text. Concentrations of $SrCl_2$, $BaCl_2$, or $MgCl_2$ when used without $CaCl_2$: 10.5 M $\times$ 10^{-4}; but 32.4 M $\times$ 10^{-4} when used with 8.1 M $\times$ 10^{-4} $CaCl_2$.

ber of isometric contractions is less than 5 per cent. After a few records the tensions became identical, which is indicated in figure 1 by one graph instead of two. The minima, i.e., the tensions at the end of each stimulation period, also show a very good agreement and even identical tension from the 4th to the 15th record (lowest curve of fig. 1). The method used in the experiments described in this paper seems to be almost ideal for the study of substances which may affect the course of muscle fatigue. Differences of even less than 10 per cent are absolutely significant!

RESULTS. I. *The calcium effect in delaying fatigue in reference to the metabolism of muscle.* A number of experiments were performed in which the influence of a higher $CaCl_2$ concentration of Ringer's solution was studied. Regularly fatigue was delayed as indicated in figure 2 when the calcium effect was studied in oxygenated solution (11 experiments). It was also determined in muscles which worked under anaerobic conditions. For this purpose the solutions contained M/1,000 KCN and no oxygenation was used. An example is reproduced in figure 3 which shows the rapid drop in the height of contractions in the control (Ringer) muscle while the "calcium-muscle" maintains the greater part of the original tension throughout the whole experiment. From these two groups of

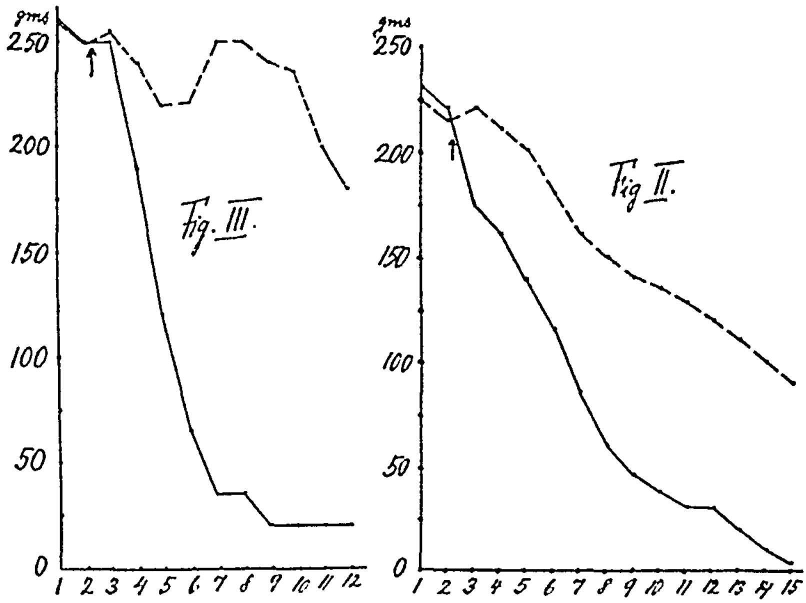


Fig. 2. The first two contractions in oxygenated Ringer's solution, thereafter one muscle (————) in the same solution ($CaCl_2$ content 8.1 M × 10^{-4}), the other muscle (----------) immersed in Ringer's with 40.5 M × 10^{-4} $CaCl_2$.

Fig. 3. Experiment in the presence of M/1,000 KCN. Designations as in figure 2.

experiments it may be concluded that the calcium effect is independent of the oxydative recovery process since it occurs in muscles which are supplied with oxygen as well as in muscles which on account of the poisonous effect of KCN cannot utilize oxygen.

In 1930 Lundsgaard showed that muscles which were previously poisoned with monoiodoacetic acid or other halogen substitutes of acetic acid do not produce lactic acid during contraction but are able to contract and to develop tension for a certain length of time before rigor occurs. The only source of energy is phosphagen under these conditions. Although Meyerhof (1931) seems to succeed in reconciling his and A. V. Hill's (1926) ideas concerning the energy changes in muscle with these new findings, it

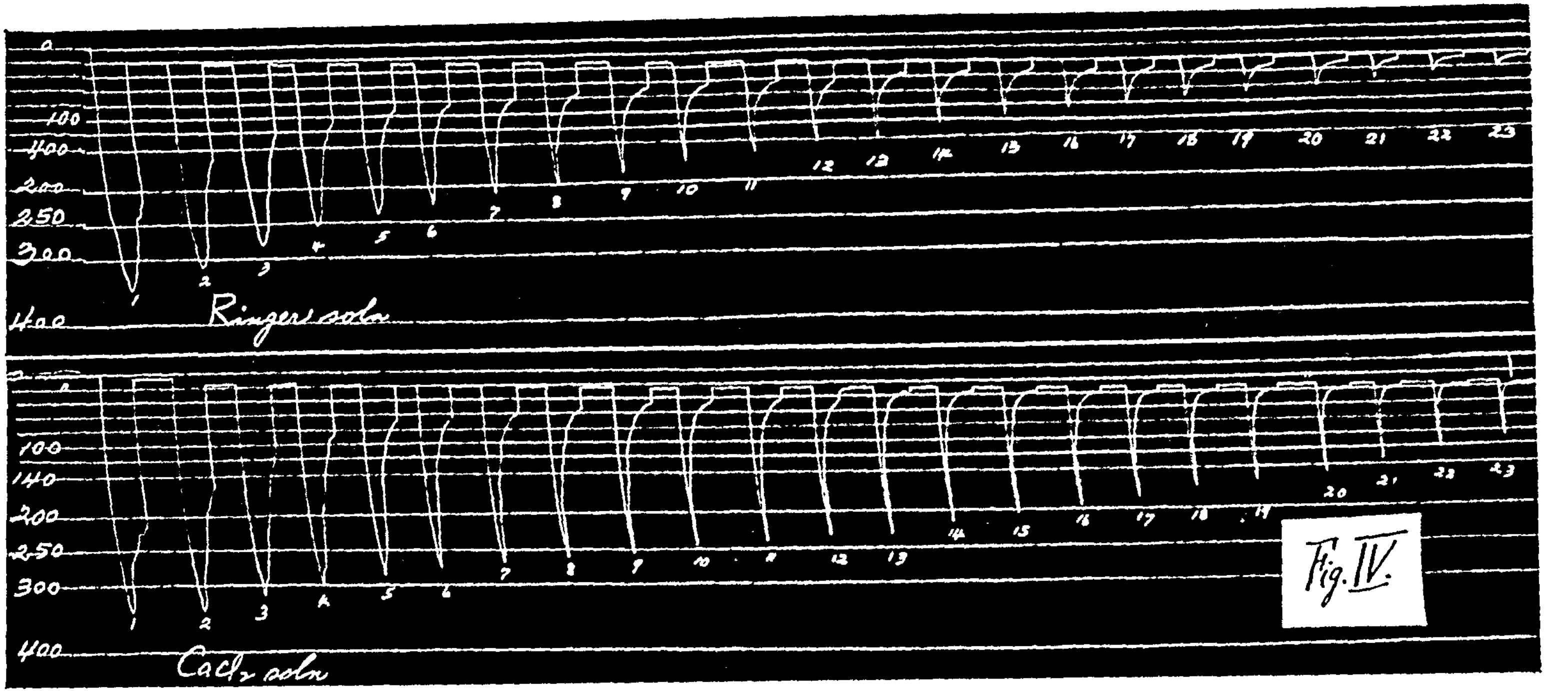


Fig. 4. Muscles poisoned with 0.05 per cent monobromoacetic acid for 30 minutes. Upper curve: Ringer's solution with 8.1 M $\times$ 10^{-4} $CaCl_2$. Lower curve: Ringer's solution with 40.5 M $\times$ 10^{-4} $CaCl_2$.

cannot be doubted that the whole metabolism of the muscle in its anaerobic part is greatly altered. Therefore the poisoning of the muscles with halogen substitutes of acetic acid seemed to be an appropriate procedure to decide the question of whether the calcium effect is dependent on certain phases of the metabolism of muscle or not. The method of Wright (1931) was used. The preparations were placed in Ringer solution containing 0.05 or 0.1 per cent monobromoacetic acid for 30 minutes at 14°. According to Wright the formation of lactic acid is thus completely interrupted. Eleven experiments were performed in this fashion and showed consistently that a strong calcium effect in delaying fatigue also occurs under these conditions. One example is reproduced in figure 4. This curve showed several other interesting data besides the marked difference in the tension maxima. Both muscles show a very gradual increase in contracture which amounts to about 40 grams tension at the end of the experiment. No significant differences in the development of this contracture, which is, of course, due to the action of monobromoacetic acid, were observed in "Ringer" and in "calcium-Ringer" muscles.

Furthermore the curves also show the "tension minima," i.e., the tension produced at the end of each stimulation period. They agree very closely in the beginning of the experiment but later significant differences occur, the minima being smaller in the "calcium" muscle than in the control muscle. It was frequently observed that an excess of $CaCl_2$ added to Ringer's solution brings about smaller minima in spite of the greater maxima which regularly occurred. This means that although calcium improves recovery and delays fatigue in this way under isotonic and isometric conditions it is not favorable to maintaining tension.

The essential fact proven by these experiments is that the delay of fatigue by calcium occurs in muscles independent of whether oxygen consumption is allowed or prevented and also independent of the formation of lactic acid during contraction. Therefore it is certain that the calcium effect is not caused by changes in the recovery process. The question of whether the calcium effect has to do with the anaerobic metabolism of muscle except that of lactic acid formation is undecided yet and may be examined in another paper. That ion effects change the energy production has been shown by Sereni (1925), but this experimental procedure is too different from ours to allow any conclusions. Although this aspect of the problem is still unsolved, the remarkable independence of the calcium effect of changes in muscular metabolism makes it highly probable that at least an important part of this effect is purely physico-chemical in nature and is restricted to its influence to reduce permeability in fatigued muscle.

II. *The effects of strontium, barium and magnesium in fatigued muscle.* In a group of experiments the question was examined as to whether Sr, Ba, or Mg might be able to replace calcium in its effect of delaying fatigue.

All experiments were negative since these ions in concentrations similar to those used in the calcium experiments were very harmful to the muscle and increased fatigue greatly. But when the chlorides of these ions were added to a Ringer solution which contained the same $CaCl_2$ concentration as the control, they showed a remarkable effect in delaying fatigue. One example of each of these groups of experiments is reproduced in figure 5. The maximal tension set up in each indirect stimulation of the muscle immersed in Ca-free Ringer's solution $+SrCl_2$ or $BaCl_2$ or $MgCl_2$ is expressed in percentage of the tension of the corresponding muscle immersed in Ringer's solution. The figure again shows the very close agreement in tension set up by two homologous muscles in Ringer's solution at the beginning of the experiment. A distinct fall in tension occurs when this solution is exchanged for another one which contains the other alkali earths instead of calcium, but an enormous rise of from 100 to 300 per cent takes place when these salts are added to ordinary Ringer's solution. The results check very well with those obtained in isotonic contractions with direct and indirect stimulation (Gellhorn, 1930–1932) but the differences are far greater due to the greater sensitivity of the method employed in the present investigations. The experiments justify the conclusion that the physico-chemical processes evoked in muscle in direct and indirect stimulation are the same provided that supramaximal stimuli are avoided. This conclusion is in agreement with observations of Hartree (1929) who found an identical heat production in directly and indirectly stimulated muscles. Therefore the generalization of Winterstein (1931) as to the unphysiological nature of direct electric stimulation is to be rejected.

SUMMARY

Isometric contractions were recorded in nerve muscle preparations of *Rana esculenta* which were stimulated indirectly by just maximal faradic currents until fatigue occurred. Stimulation periods (5–30 sec.) and rest periods (2–5 min.) alternated. Under these conditions two homologous muscles of the same frog give almost identical tensions.

A Ringer's solution containing an excess of calcium delays fatigue greatly. This phenomenon is independent of fundamental changes in the metabolism of the muscle since it is obtained in oxygenated solutions, in the presence of KCN which suppresses the utilization of oxygen, and after poisoning with monobromoacetic acid (suppression of lactic acid formation). The experiments seem to indicate that the delay in fatigue is chiefly due to physico-chemical alterations of the surface layer of muscle cells (decrease in permeability) although an influence of calcium on the anaerobic phase of muscular metabolism is possible.

When $CaCl_2$ is replaced by equimolar concentrations of either $SrCl_2$ or $BaCl_2$ or $MgCl_2$, fatigue is greatly enhanced. But these salts bring about

a beneficial effect on the muscle (delay in fatigue) which is comparable to the calcium effect described above when they are added to an ordinary Ringer's solution containing 8.1 M × $10^{-4}CaCl_2$.

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VAGAL DEPRESSION OF THE TURTLE ATRIUM

A Study of the Integration of Effect of Nerve Impulses[1]

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The phenomena which are as a group referred to as vagal cardio-inhibition offer typical examples of summation of effect of nerve impulses. We have selected one aspect of the vagal cardio-inhibitory complex for quantitative study. This aspect, the negative inotropic effect (depression of strength of contraction) on the atrium may usually be observed following the application of a single stimulating shock to the vagus nerve in the neck. The reduction in strength of the atrial beat following such a single vagus volley does not appear immediately in its fullest extent but continues to increase over a period of several beats, attains a maximum, and then gradually subsides. The intensity of the effects at any time and the differences which appear when shocks of various strengths are used may be studied from measurement of kymograph records. Such records usually show a slight amount of atrial depression in consequence of a weak shock and a much greater depression resulting from the effects of a stronger shock.

Now, according to the all-or-none concept, an increase in amount of cardiac effect due to an increase in the strength of a single shock applied to the nerve can only mean that more nerve fibers have been stimulated by the stronger shock. Thus, the amount of atrial depression is related to the number of nerve fibers which have been made active. In so far as the nerve response is concerned, the limits of this change of effect must be determined by the threshold shock strengths necessary for stimulation of the most irritable nerve fiber and of the least irritable nerve fiber respectively in that nerve bundle supplying the organ under observation. With respect to the response in the heart, the least effect to be observed will depend largely upon the sensitivity of the method used for recording, whereas the maximal effect produced will be that developed by the simultaneous activity of all the fibers in the nerve bundle. Such a consideration presents the implication that, in an organ showing the typical phenomena of summation of nerve impulses, the total amount of effect produced should depend upon the number of nerve fibers active during a certain period of

[1] The author wishes to express his thanks to Dr. Frank Urban for assistance in the mathematical analysis of the experimental material.

time. The same total effect would then be produced whether many nerve fibers were stimulated a few times or a few fibers were stimulated many times. The maximal effect of several nerve volleys or the intensity of the effect at any time should depend upon the number of fibers active in each volley, the number of volleys discharged in a unit of time, and the time course of the effect produced by a single volley.

It was found that, with a given preparation, if the figures obtained from a series of records were plotted on a cartesian coördinate system, the percentage amount of atrial depression being plotted on the ordinate and time on the abscissa, curves of a regular form could be obtained. The curves from different records might show maximal depression values of different amounts, but the time values of the curves were surprisingly constant. That is, although the percentage depression in height of the atrial contraction record following the application of a weak shock to the vagus might be much less than that following a strong shock, the time to maximal depression and the time course of the recovery process were, respectively, almost identical in the two records. The use of such curves offered a means of studying summation phenomena as they are expressed in vagal cardio-inhibition. The material presented below is a report of the results obtained from certain experiments on the vagal inotropic effect on the turtle atrium. It is based on a study of records in which stimulation of the vagus did not produce significant changes in the spontaneous rhythm of the heart.

Material and methods. The effect of stimulation of vagus cardio-inhibitory fibers varies somewhat with the seasons of the year. At a given time, however, turtles from the same lot show considerable regularity of response. This is particularly true of the atrial inotropic effect (depression of contraction) which results from application of single shocks to the vagus nerve in the neck.

Turtles, usually eight inch specimens of Pseudemys elegans, were used. Other species gave similar results. In preparing for an experiment, the brain of the turtle was pithed, the turtle fixed on a board, plastron removed, fore legs removed, vagi exposed, dissected free from the artery and surrounding tissue, and cut high in the neck, sympathetic nerve on each side cut near its junction with the vagus, pericardial chamber opened widely, ventricle cut away, and a light spring lever attached to one auricle by a light wire pinzette. In most of the experiments the total tension of the lever during diastasis corresponded to that exerted by a weight of about one and a half grams. A maximum of half a gram additional tension was required to draw the lever down to the position occupied at the peak of atrial systole. In all the records reproduced, contraction of the atrium was represented by a downward movement of the writing lever. Magnification of movement due to leverage was about five times. Stimulation

of the nerve was accomplished by laying the nerve on silver wire or silver chloride electrodes and subjecting the nerve to a shock produced by the charging of a condenser. Records made by use of the cathode ray oscillograph with adequate amplifying and spreading devices showed that with the shock forms and strengths used, a single shock resulted in only a single volley of impulses being sent along the nerve. Repetitive stimulation of the nerve occurred therefore only when the key was repeatedly opened and closed.

Stimulation of the left vagus usually produces less change in the rate of the turtle heart than does stimulation of the right vagus (see Garrey, 1911). Therefore, in order to insure records showing inotropic changes with no rate changes (chronotropic effects), the left vagus and left atrium were usually used. Such a procedure is, however, essentially one of convenience, giving an increased available range of shock strength. With shocks of short duration, it is usually possible to obtain pure inotropic effects in the turtle heart with weak stimulation of the right vagus just as it is also generally possible to slow or stop the heart with sufficient (usually repeated) stimulation of the left vagus.

Kymograph records were made and were measured either directly or by projection giving magnification of about five times. The records showed a maximum of excursion of the writing point of about 20 mm.

Experimental findings. a. *The time course of the inotropic depression following a single stimulation of the vagus nerve.* It was recognised by earlier workers in the field that a single shock applied to the vagus does not produce its maximum inotropic effect immediately after the completion of a period of latency, but only after one or several heart beats have occurred (see fig. 2). The time to maximum depression in a given preparation under constant conditions is quite constant as is also the curve of recovery following the attainment of that maximum. After a preparation has been under observation for some time, the recovery process tends to become slower, but at any time a change in effective strength of the vagus stimulation will result in increased or decreased inotropic effect without markedly changing the time functions of the curve. Records taken at a drum speed of about 20 mm. per second were measured and showed that the curve of depression plotted against time begins to rise slowly. The slope then increases but attains a maximum only several tenths of a second after its start (fig. 1, curve *2*). This result was invariable and therefore puts the curves outside the family which may be represented by the general equation

$$y = m\,[e^{-a(t_1 - t_0)} - e^{-b(t_1 - t_0)}] \qquad (1)$$

A curve of this form is plotted as curve 3 of figure 1.

In the light of the various chemical theories of inhibition which have been brought forward, it seemed not unreasonable to try to fit the curves plotted from our records to calculated curves obtained as a result of the following argument. Suppose the immediate peripheral effect of a single nerve fiber impulse to be the liberation of an inhibitory substance in concentration n. To produce its depressant effect this material must be diffused through a distance x. Moreover, since the recovery of the heart from depression is rather prompt, suppose the material to be destroyed at a rate proportional to its concentration.

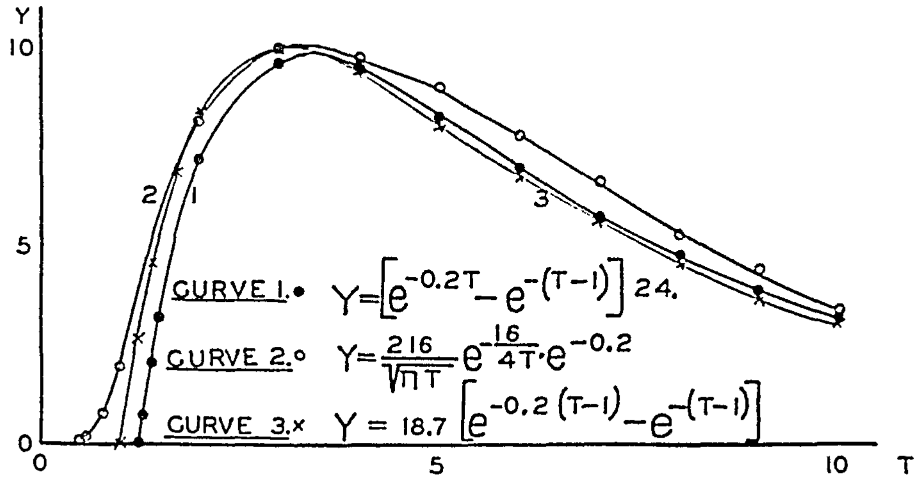

Fig. 1. Curves plotted from the three equations presented. Curve *1*, plotted from equation (5) developed by Bremer and Homès. Curve *2*, plotted from equation (4) and fitting the course of atrial depression following a single vagus volley. Curve *3*, plotted from equation (1) for comparison.

To describe the element of diffusion, we used the simple equation given by Eucken (1931).

$$C = \frac{n_0}{q\sqrt{\pi D t}} \cdot e^{-\frac{x^2}{4Dt}} \qquad \text{(2)}$$

As we have employed the equation in plotting curves, n_0 represents the total amount of a diffusible substance having the diffusion coefficient D. At time zero, this amount (n_0) is considered to be placed in a cylinder of infinite length and of cross section q. The substance is located within a layer of volume qdx at the region $x = 0$. Diffusion then begins and is here considered in the positive direction only. The value of c represents the concentration of the substance at time t in a layer of volume qdx which is located at a distance x from the origin.

The solution of the problem of the concentration at time t of such a diffusing substance which is being chemically destroyed at a rate propor-

tional to its concentration may be found in the recent paper of Miller and Gordon (1931). The equation giving the solution of this problem is

$$y = ce^{-kt} \qquad (3)$$

where c is the value given by equation (2) and k is the velocity constant for the chemical process.

We obtained, therefore, a final equation

$$y = \frac{n_0}{q\sqrt{\pi Dt}} \cdot e^{-\frac{x^2}{4Dt}} \cdot e^{-kt} \qquad (4)$$

Arbitrary values were substituted for the constants of equation (4) and a series of empirical curves plotted. Certain of these curves were found to fit, with considerable agreement, the curves plotted from the experimental data. Such an empirical curve is plotted as figure 1, curve 2.

As will be seen by reference to the figures, the curves resemble, in general, skew distribution curves. Therefore, although the curves developed on the basis of an assumed chemical process show fair agreement with the experimental data, too great emphasis is not to be placed on this phase of the work. We can say definitely that stimulation of certain fibers in the vagus nerve produces an effect which achieves its maximum only after a period of time corresponding to several cardiac cycles. Furthermore, the achievement of this maximum and the course of the ensuing recovery occur along a definite depression-time curve.

b. *Effects of change in shock strength upon the depression curve.* Below a certain threshold strength of the shock applied to the vagus no inotropic effect is produced in the atrium. As the shock strength is increased, single shocks to vagus result in atrial inotropic changes which increase from a just visible minimum to a maximum which is not surpassed by further increase in shock strength. In a given preparation, the depression-time curves from several successive records may change in height but do not change in form with respect to time. If the ordinate (percentage depression) values of each curve of such a series be multiplied by a factor such as to bring them all to the same plotted maximum, they become practically confluent (fig. 2). One reason for such lack of agreement as is found is discussed in section d.

c. *Effects of repeated vagal stimulation.* If a shock strength be selected such that a single shock produces a just measurable inotropic effect on the atrium, repeated stimulation at sufficiently brief intervals will produce an increased effect. A series of records showing this is seen in figure 3, records G to L inclusive. The records show the effects of stimuli numbering 1, 2, 4, 8, and 1 respectively. The shocks were applied at intervals of about one-fifth of a second. The ratio between the percentage of depres-

sion and the number of shocks decreases somewhat as the number of shocks increases, the effect of the eight shocks being slightly less than eight times

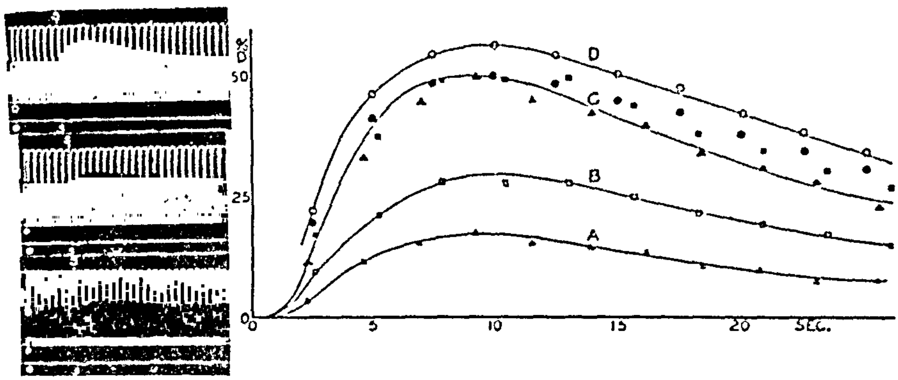

Fig. 2. Curves A, B, and D plotted from the kymograph records reproduced. Ordinates, percentage depression of the height of the atrial contraction as recorded. Abscissae, time. Curve C, blacked-in symbols represent points from A, B, and D, each set being multiplied by a factor to bring all to the same maximum. The curve drawn was plotted by substituting in equation (4) the values $n_o = 71.5$, $q = 1$, $D = 0.5$, $k = 0.05$, $x = 4$. Shocks used in each case were obtained by charge of 0.1 mfd. condenser. Voltages for A, 13.5; B, 15; D, 22. Kymograph records in this and other figures, from above downward, each show atrial contraction (systole gives downward movement), time in seconds, and moment of stimulation of vagus nerve in the neck (downward movement of signal).

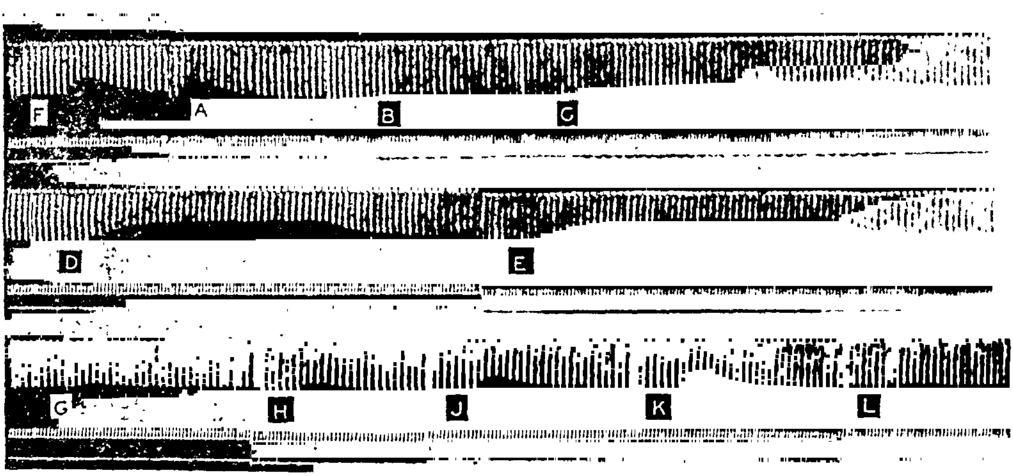

Fig. 3. A to F inclusive experiment 2/14/32, recorded in sequence as lettered. 0.1 mfd. A and F single shock, 22 volts; B, C, D and E 6 volts; B single shock, C, D, and E stimuli at intervals of 3 seconds, 1.5 second, and 0.75 second respectively. G to L, effects of 1, 2, 4, 8 and 1 shocks respectively; shock intervals about ½ second.

the depression produced by one shock. The deviation is more than would be expected merely as a result of temporal dispersion of the shocks and will be considered further in section d.

Stimulation of the vagus at moderate rates (from one shock in several seconds to about twenty shocks per second) will cause development of a summated inotropic effect to a certain equilibrium level which is then maintained. If the stimulation is continued until this level is reached, it is often found that the time course of the recovery is apparently slightly slower than normal (see section d).

With a given rate of stimulation, the maintained level of depression increases between threshold and maximal as the shock strength is increased. In many experiments with stronger shock strengths, the maximal effect which might be obtained is masked by the appearance of rate and conductivity changes. The complications brought in under such conditions are not considered in the present paper. Using a given strength of stimulus, increase in stimulation rate, up to a certain limit, results in an increased level of depression (fig. 3, records A to F incl.). This aspect of the subject is considered at some length in the earlier literature.

d. *Lack of rectilinear relationship between the number of nerve impulses and the inotropic effect.* The records and corresponding curves of figure 2 were selected for reproduction because they demonstrated clearly a phenomenon which has been neglected in the previous sections. It is not strictly true that the maximal percentage depression following two vagus nerve volleys is twice that resulting from one volley. Successive increments of vagal impulses apparently do not produce arithmetically graded steps in the atrial depression record but rather steps the height of which seems to be related to the height of the atrial contraction at that moment. Therefore, as the depression increases, successive vagal increments produce less and less effect and there is increasing deviation from a rectilinear relationship between the number of vagal increments and the degree of depression.

We believe this to be a reason for the apparent differences in recovery curves found when comparing records obtained 1, with single shocks of different strengths, and 2, with lesser and greater degrees of inhibition during periods of continued stimulation. The effect of this lack of a rectilinear relationship is seen in figure 2 and in records C, D, and E of figure 3.

In figure 2, curve C was plotted from the equation (4). The three sets of blacked-in symbols represent the plotted symbols of the curves A, B, and D but with each group of values multiplied by a factor such as to bring the four curves to the same maximum. The curves therefore necessarily have the same maximum height. Elsewhere, however, the circles, from curve D, consistently lie above the triangles, from curve A. A similar effect is seen in figure 3, records C, D and E. The intervals between stimuli applied to the vagus were respectively 3, 1.5, and 0.75 seconds. Complete equilibrium levels of depression were not quite reached but, at the time the

stimuli were discontinued, the percentages of depression achieved were respectively 18, 36, and 42. The curves of recovery plotted from such records would thus tend to show an apparent decrease in the value of k of equation (4) as the maximal level of depression increased.

e. *Effects of temperature.* Records made at different times of the year and under sufficiently controlled conditions have not yet been obtained in sufficient numbers to allow of definite statements as to seasonal changes in the nature of the atrial inotropic effect. The impression gained from the records available is that this effect is greatest and proceeds with greatest speed in animals in the late spring, being the least and slowest in the late fall or winter. Such variations seem to introduce complications into a study of temperature effects. The immediate effects of temperature changes were investigated on a series of turtles during November and

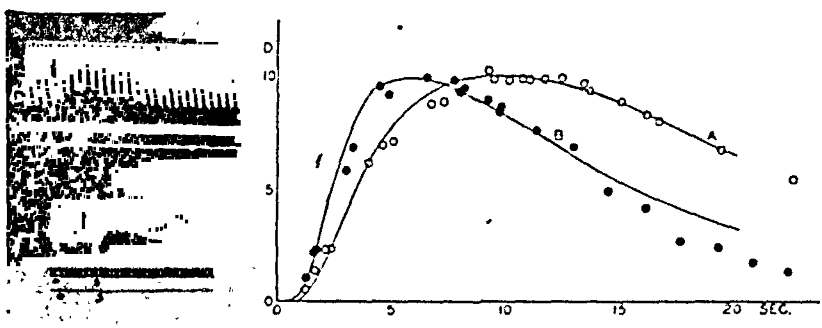

Fig. 4. Effects of single vagus volleys at different temperatures. Experimental values plotted from several records of which two are reproduced. Depression maxima brought to same level for plotting. Record A, curve *A*, 16°C., solid line plotted from equation (4), D = 0.5, k = 0.05. Record B, curve *B*, same preparation, 22°C., D = 0.75, k = 0.08.

December. Animals were brought from the cool storage pen in the basement and kept for two days in the laboratory in running water at about 14°C. Before beginning an experiment, the laboratory temperature was brought down to between 12°C. and 15°C. An animal was then prepared and a series of records taken. The laboratory temperature, and with it the temperature of the turtle, was allowed to rise over a period of one or two hours and another series of records was taken (fig. 4). The temperature of the room and of the turtle viscera at that time would be between 20° and 23°C. This last series of records yielded curves which were similar to those obtained from turtles kept in the warm laboratory (at about 23°C.) for two days before an experiment done at a temperature of about 23°C. and were "slower" than curves obtained at similar temperatures in April or May.

In all cases, as the temperature rose, the times to maximum inhibition

and to any definite percentage of recovery became less. When the curves obtained were studied with respect to the rate of recovery from inhibition, it was found that the change appeared to correspond to changes both in D and in k of equation 4.

The change in rate of the heart in response to the temperature changes was such that the number of beats occurring between the application of the single shock to the vagus and the moment of maximal inhibition was quite constant. Records taken during the summer with temperatures up to 38°C. showed a similar situation. It appeared thus as if there might exist a causal relationship between the number of heart beats and the course of the inhibitory process. That such is not the case was revealed by the series of experiments reported below.

f. *Effect of changes in heart rate.* If an intact heart be driven by means of electrical stimuli applied to the atrium at a rate barely above that of

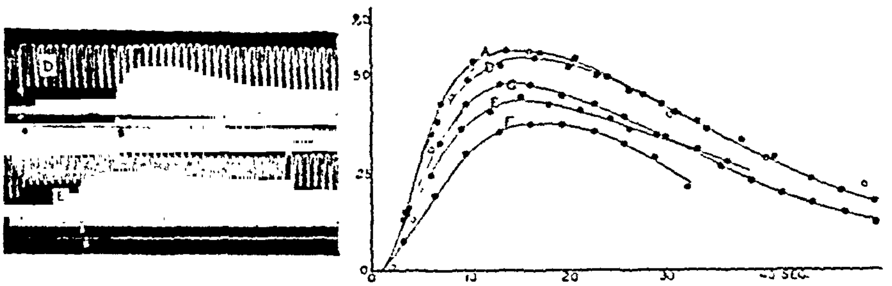

Fig. 5. Effects of single vagus volleys on atrium responding at different rates. Plotted curves A, D and G from spontaneously beating heart, beat interval 3.2 seconds. Curves E and F from same preparation, heart driven, beat interval 1.5 seconds. Records from which D and E were plotted are reproduced.

the spontaneous rhythm of the heart, there is usually some evidence of vagal stimulation of the vagus fibers in the heart. It can be shown, however, that a single shock to the vagus applied during such a time will produce an atrial depression curve of the usual form. If the atrium be driven by means of stimuli applied through a fine wire hooked into the tip of the auricle, the circuit being completed through an indifferent electrode buried in the viscera, the intra-cardiac vagal stimulation is probably at a minimum. Using such an arrangement records were made from several hearts which were driven at rates of from 1.6 to 2.2 times that of their spontaneous rhythms.

The driving stimuli were begun and when a fairly constant contraction height was reached, a single shock was applied to the vagus. The resulting atrial inotropic depression curve was of the same form as the control depression curves obtained from the same hearts beating spontaneously.

Results from such an experiment are presented in figure 5. Two records are shown, showing the effect of single shocks to the vagus in the first case, *D*, with the heart beating spontaneously with a beat interval of 3.2 seconds, the other, *E*, showing the heart driven with a beat interval of 1.5 seconds. Because of the alternating irregularity of the driven atrium, contraction heights from records *E* and *F* were averaged in pairs and the figures so obtained where plotted. The curves *A*, *D* and *G* were plotted from records of the spontaneously beating heart; curves *E* and *F* are plotted from records of the driven heart.

The mere number of atrial beats is, therefore, in no causal way connected with the duration of depression following a single vagus volley. In all the records obtained from driven hearts, however, the strength of the atrial beat was less than that of the slower, spontaneously driven atria. The possibility that a study of the total work or total tension developed might show some correlation with the course of recovery has not as yet been experimentally investigated.

Discussion. We have previously (1931) pointed out that for the turtle heart, vagus standstill does not represent a threshold chronotropic effect. Standstill, although it represents a fairly definite degree of inhibition, occurs only with the development of chronotropic and dromotropic effects which are considerably above threshold. With the case of the inotropic effect of the vagus also we do not believe that we can recognize any definite or absolute criterion of threshold effects in the heart.

Heinbecker (1931) has shown that the appearance of a definite component of the turtle vagus action potential is correlated with the onset of the inotropic effect in the atrium. It is, however, probable that the potential contributed by the first fiber (or the first few fibers) could not be recognised from the oscillograph records as yet obtained. Where optimally rapid stimulation, continued "indefinitely" can be used, as is generally the case, measurements of the atrial contraction record is undoubtedly a more delicate method than either the form of the action potential or the measurement of the concomitant changes in the heart (shortening of systolic time, shortening of the absolute refractory period, shortening of chronaxie, and depression of the action potential). It is for this reason and because it offers the least technical difficulty that we used measurement of the atrial contraction record in this study.

The form of the depression-time curves which we have obtained leads to two obviously possible modes of vagus action in the heart. One is that the curves are distribution curves representing delays in the different elements of the cardiac ganglionic or post-ganglionic mechanism. This delay system would be such that a maximum number of nerve elements are effectively active on the cardiac musculature only after the completion of an apparent latency of several seconds. Another possibility is that the

development of the empirical curves according to a chemical hypothesis is in some agreement with the actual facts.

The former of these alternatives, that of a delay mechanism, would mean the presence, in the intrinsic cardiac nerves, of an activating system similar to that assumed by Eccles and Sherrington (1931) to occur in the spinal cord. Such an assumption would demand that the intracardiac nerve path should be so intricately arranged as a conducting, integrating, and facilitating system within itself that, although the effect on the cardiac musculature of a single shock to the vagus was not at its maximum for ten or more seconds, every activated locus along that path must have recovered from refractoriness in a tenth of a second or less. Were this not true, it would not be possible to observe the degree of summation actually obtained when ten or more shocks per second are applied to the vagus. The time required for development of maximal depression, following a single vagus volley, is so great that we doubt whether such a delay circuit offers an adequate mechanism.

The second obvious possibility of a mode of vagus action involves the existence of an inhibitory mechanism which acts on the contractile mechanism indirectly as through the intermediation of a chemical system. The necessity of using arbitrary values for the constants of equation (4) must leave those values without present significance as to the component details of any physico-chemical system which might be involved. Moreover, even using a simplified equation, the two of these constants which would be most desirable are not at present available. These are first, the diffusion coefficient for the hypothetical substance in question and, second, the distance through which it would need to diffuse to produce its action. Although our experimental results might seem entirely compatible with a specific hypothesis such as that developed by Loewi (1926, etc.), a variety of other, quite different, chemical systems would yield similar action-time curves. Changes in the post-ganglionic cell body or in the muscle fiber might be produced by the immediate effect of a nerve impulse and might also develop and disappear in the manner observed.

For the present we must restrict ourselves to the statement that our evidence demonstrates that stimulation of a single nerve unit (fiber or small group of fibers stimulated in the neck region) produces a change in the atrium. This change develops to a maximum and disappears in accordance with a definite intensity-time curve. An increase in the number of nerve units activated within a unit of time may be shown experimentally to produce just such an increase in effect as would be expected. The increase in the number of nerve units activated per unit of time may be brought about by an increase either of shock strength or of frequency of stimulation.

Moreover it is probable that such an interpretation of summation phe-

nomena can be applied to the effect of nerves in all organs in which a single summated effect can be obtained in consequence of an integration of nerve impulse effect. When the problem is viewed in the light of such a consideration, one encounters difficulty not in naming nerves which have "iterative" properties but rather in naming those nerves which do not have such properties. Lapicque has considered as "iterative nerves" those which must be stimulated several times in order to produce a threshold response of their end organ. It seems probable that in all such cases, the level at which the "threshold" response is produced depends upon the sensitivity of the recording system used. Among the nerves classed by Lapicque and his co-workers as iterative are sensory fibers, the vagus to the heart (producing standstill in the frog) the vagus to the stomach, and the splenic nerve causing contraction of the spleen (see Fredericq, 1928). Bishop and Heinbecker (1932) report typical summation phenomena resulting from stimulation of the cervical sympathetic nerve in cats and rabbits. They find that such effects are observed in the nictitating membrane, the pupil, blood vessels, and erectile hairs. Although summation may be seen in all of these, some of them show measurable response after a single shock to the nerve while others show a measurable response only when several shocks have been applied.

Even the motor nerve fibers to skeletal muscle appear under certain conditions to give an integrative effect. Bremer and Homès (1932) have published a theory of summation of the nerve impulse which is an extension of work reported some years ago by Bremer. They make use of a phenomenon observed by Boehm (1894). Boehm found that a muscle from a lightly curarised animal might fail to respond when the motor nerve was subjected to a single electrical shock but that when rapidly repeated stimulation was used, the muscle might contract. Bremer and Homès studied the effect of two shocks applied to the nerve in rapid succession. Their hypothesis assumes that the shock to the nerve be applied sufficiently close to the muscle so that transmission time becomes negligible. Following the application of a first shock to the nerve there is produced a change in the neuro-muscular junction in consequence of the arrival there of the nerve impulse (*influx nerveux*). This change may or may not be sufficient to produce a response of the muscle and is dissipated spontaneously as along the curve of a decreasing exponential. A second shock applied to the nerve during the absolutely refractory period following the first shock, produces, of course, no measurable effect at the neuro-muscular junction. However as the shock is applied later, there will be an increasingly greater effect produced at the junction. The curve of this increasing effect is supposed to be an increasing exponential for which the level of complete recovery is the asymptote. The total or summated effect at the neuro-

muscular junction is determined by the sum of the two effects. The strength of the muscle contraction should be an expression of this summated effect and the recorded contraction heights from twitches produced by paired shocks, separated by different times, should follow the curve defined by their equation

$$R = \alpha\,[e^{-at} - e^{-b(t - t^*)}] \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (5)$$

where t^* represents the time at the end of the absolutely refractory period. Bremer and Homès publish myographs which they consider to be in agreement with this argument. Their plotted points have, however, been so crowded along the time axis that there is a masking of the gradually rising start which is clearly present in nearly all of their published records. We believe that their figures can be fitted better by curves of the form similar to those from equation (4) than by the equation which they develop. It is perhaps not impossible that a temporal distribution due to different recovery times at different junctional points introduces distortion into curves which might otherwise agree with the argument which they offer.

It seems probable that, in any tissue which shows summation of nerve impulse effect, there is a considerable time range during which summation of two or more nerve impulses can be effected. The least time during which any such effect could be produced will depend upon times of local refractoriness in any part of the system. The longest time through which summation effects can occur will be determined by the effective latency of action of the system and the duration of the effect produced by a previous impulse. A system limited in time only in this way and in which the all-or-none law at least in simple form, does not hold, should show summation phenomena in just such a way as they usually occur. Summation of sub-threshold stimuli can probably occur in any tissue. In a tissue following the all-or-none law and in which achievement of a definite threshold level (of excitation) is followed by a specific response and an ensuing period of refractoriness, the reaction has probably become complicated but not essentially changed.

Our experiments make it seem probable in the case of vagus activity in the heart as in other systems giving integration of nerve impulses, that emphasis should be placed not on the minimal but rather on the total time during which summation effects may be developed. The essence of such a concept is contained in Lapicque's (1925) theory of latent addition, in the work of Bremer, and of others. It appears, however, that an hypothesis which becomes too highly specific in detail, either as to threshold conditions or as to mechanism of the responding system is not as yet warranted by the experimental facts available.

SUMMARY AND CONCLUSIONS

Vagal stimulation in the turtle was studied with respect to the time course of the depression of atrial contraction following such stimulation. When a single effective shock was applied to the vagus, the consequent inotropic effect in the atrium followed a definite intensity-time course. The plotted curves have the general form of skew distribution curves. They may, however, be satisfactorily fitted from an equation defining the concentration of a substance under conditions of both diffusion and chemical change.

Increase in strength (between threshold and maximal) of single shocks applied to the vagus resulted in an increase in atrial depression (between threshold and a maximum for single shocks) with but little change in the time functions of the curve of depression.

The times of achievement of and recovery from depression are increased as the temperature of a given preparation is lowered.

Driving the atrium by electrical shocks thrown in at rates of about twice that of the normal rhythm does not affect the time course of the inotropic depression resulting from a single shock to the vagus.

If, with a given shock strength, the depression following a single shock be compared with that resulting from several shocks, the summated effect from the latter is in accordance with that which would be expected from a study of the former. This is true both for the degree of maximal depression achieved and the time required for the achievement of the depression. If repeated stimulation be continued "indefinitely," the depression develops to a certain level which is then maintained with little or no change as stimulation is continued. Within physiological limits, this depression is increased either by keeping the shock strength constant and increasing the frequency, or by keeping the frequency constant and increasing the shock strength.

We are led to the conclusion that in the preparation used, summation of nerve impulse effects is largely determined by the intensity-time course of the effect produced by a single stimulation of a single nerve unit. Conditions of refractoriness in the system determine the minimum time which must elapse between successive stimuli if each is to be effective. The duration of the change produced by a nerve impulse determines the maximum time for summation. Within the limits of such conditions, repeated stimulation produces the expected summated effects.

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STUDIES ON THE PHYSIOLOGY OF SLEEP

VI. The Behavior of Decorticated Dogs

NATHANIEL KLEITMAN and NELATON CAMILLE

From the Department of Physiology of the University of Chicago

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For the last forty years many investigators of sleep have attempted to localize a sleep center in the nervous system. While some came to the conclusion that no such center existed, others have placed it in a variety of regions, from the cerebral cortex down to the medulla. Some recent workers, on the basis of laboratory and clinical investigations, favor the region around and at the floor of the third ventricle (the periventricular gray), a region originally shown to have something to do with sleep by Mauthner (1890). There is a group of adherents of Pavlov who, with him, interpret sleep as a generalized spreading of inhibition through the cerebral cortex. A third group tries to reconcile these two divergent viewpoints by postulating that the cortical sleep apparatus is duplicated in the lower nervous centers (Johnson, 1923), or that there is a double sleep center concerned with body sleep on the one hand and brain sleep on the other (Economo, 1928; Adie, 1926).

Reference is often made to the behavior of decorticated dogs as proving that sleep is possible in the absence of a cerebral cortex. Unfortunately those who observed the behavior of decorticated dogs made no special studies of their sleep, and that probably accounts for the divergent and even contradictory statements they made on this subject, although they are by no means unanimous in their descriptions of other aspects of behavior of decorticated animals.

Goltz (1892), the first one to keep decorticated dogs alive for any length of time, made what appear to be the most accurate observations of the sleep of these animals. In the morning he often found the dogs asleep, their eyes closed, and their breathing regular. They could be awakened, but more powerful than usual stimuli were required for that purpose. Goltz could detect no evidence of dreaming in his decorticated dogs. During their waking hours the dogs were hyperactive, walking aimlessly for long periods of time, generally in a circle. After they were fed the dogs made a few turns, then lay down and fell asleep. Their rest and sleep were usually of short duration. Deprivation of food increased the restlessness of the dogs.

Rothmann (1923) not only found that his decorticated dog slept after the manner of a normal dog, but also detected a sense of time (Zeitgefühl) in his animal. The dog slept at night and was awake during the daytime. The dog apparently also knew when he was going to be fed, because at the proper time he would of his own choice place himself by the door of his cage and wait for his food. Rothmann's dog was also hyperactive at times. The hyperactivity was especially marked before micturition or defecation, and when the dog was hungry.

Dresel (1924) kept a dog alive for three months after removing his cortex and striatum. The dog was very active before micturition, or when hungry. Unlike Rothmann's dog he manifested no regular diurnal sleep.

Rademaker and Winkler (1928) make the following statement regarding their decorticated dog "Robbie:" "The animal lived in two alternatingly varying periods. In one it was restless, it tried to make walking movements and to lift itself. In the other period it seemed to sleep. If shaken or pinched, it opened its eyes, yawned and moved its hair as a dog that is awaking." They do not say what relation, if any, to day and night the "two alternatingly varying periods" had.

Zeliony (1929) observed two decorticated dogs and noted that they slept most of the time. In one of them, "Alpha," only hunger and the need for micturition or defecation called forth awakening. He also observed that hunger made that dog pace the floor and execute chewing movements.

This exhausts our information concerning the activity and rest of decorticated dogs. All investigators seem to agree that the dogs slept after the extirpation of the cerebral cortex. But does a decorticated dog sleep most of the time, or is he awake for longer periods of time than he is asleep? Do the periods of rest and activity alternate regularly? Does such an animal manifest any sense of time, as reported by Rothmann? New observations on the effects of decortication had to be made, with sleep as the special object of study, before these questions could be answered.

Methods and results. After repeated unsuccessful attempts to keep dogs alive upon the removal of the entire cerebral cortex in one operation, the older two stage method was resorted to. The animals were operated upon under either barbital or ether anesthesia. The completeness of decortication was verified in each case by post-mortem examination. While detailed microscopic studies have not yet been made of the preserved brain stems, careful inspection of whole and sliced brain stems revealed very little or no cortical tissue. In most cases too much rather than too little brain tissue was removed. In some dogs, as a preliminary procedure, both vertebral arteries were tied off permanently and the two carotid arteries compressed during the removal of the cortex. If the animal recovered from the first operation in which the cortex of one hemisphere was removed, four to six weeks were allowed to elapse before the cortex of the

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This exhausts our information concerning the activity and rest of decorticated dogs. All investigators seem to agree that the dogs slept after the extirpation of the cerebral cortex. But does a decorticated dog sleep most of the time, or is he awake for longer periods of time than he is asleep? Do the periods of rest and activity alternate regularly? Does such an animal manifest any sense of time, as reported by Rothmann? New observations on the effects of decortication had to be made, with sleep as the special object of study, before these questions could be answered.

Methods and results. After repeated unsuccessful attempts to keep dogs alive upon the removal of the entire cerebral cortex in one operation, the older two stage method was resorted to. The animals were operated upon under either barbital or ether anesthesia. The completeness of decortication was verified in each case by post-mortem examination. While detailed microscopic studies have not yet been made of the preserved brain stems, careful inspection of whole and sliced brain stems revealed very little or no cortical tissue. In most cases too much rather than too little brain tissue was removed. In some dogs, as a preliminary procedure, both vertebral arteries were tied off permanently and the two carotid arteries compressed during the removal of the cortex. If the animal recovered from the first operation in which the cortex of one hemisphere was removed, four to six weeks were allowed to elapse before the cortex of the

other hemisphere was taken out. After each operation, but especially after the second, most of the animals were in a state of profound depression for some days, unable to stand up, to eat or to drink. Four dogs lived long enough after the second operation to permit satisfactory studies. Dog A lived for 158 days, B was killed one year after decortication, and C and D lived only for 17 and 14 days respectively. Each of the latter two dogs, however, made a very quick recovery after the second operation (C walking well on the next day) and was studied intensively, when he suddenly took a turn for the worse and died. Autopsy showed no evidences of infection in either case. We would hesitate in drawing conclusions from observations made on dogs C and D alone, but since they behaved in every way like the longer lived dogs A and B we feel that we are justified in considering the results obtained on all four dogs as valid.

Even casual inspection left no doubt that decorticated dogs slept. They lay curled up or sprawled on the floor, eyes closed, and breathing quiet and regular. Upon awakening they nearly always stretched and yawned. It was easy to follow the various stages of drowsiness preceding sleep. Through the semiclosed eyelids one could observe how the eyes rolled downward and outward, and the nictitating membranes crept out and partially covered the eyes. Occasionally when nearly asleep the dogs made chewing movements repeatedly. That woke them up, and each time it happened the eyes returned to the normal position. During the time they were sleep the eyes remained rolled outward and downward, as could be seen by gently spreading the eyelids. There was a definite lowering of reflex irritability with the onset of sleep. In the waking state, the dogs lying flatly on the floor, an auditory stimulus of a constant intensity and high pitch elicited a stereotyped response a great number of times in succession. This consisted of a lifting of the head and a movement of the ears, the "investigatory reflex" of Pavlov. In normal dogs the investigatory response gradually fails upon repeated stimulation, but not so in decorticated animals. This fact was already noted by Zeliony (Pavlov, 1927), and we were able to confirm this observation. However, if the stimulus is applied every ten seconds, while the decorticated dog is falling asleep, the response becomes feebler, may follow one stimulus and not the next one, and finally completely dies out. As in normal dogs, after the onset of sleep, a response can be obtained either by applying the stimulus more frequently, or by increasing its intensity.

Once asleep the dogs did not wake up spontaneously for periods of time varying from 30 minutes to several hours, but most frequently they slept from one to two hours. In the waking state they generally walked about, sometimes incessantly, sometimes with short periods of rest, for a long time, as observed first by Goltz, and later by others. As a rule, the walking was in a circle. We utilized this fact in making the dogs record their

activity by means of a simple device. A hand centrifuge was suspended in the air, upside down, about three feet from the ground. A four foot rope connected the well lubricated axis of the centrifuge head with the dog's collar. The dog's walking in a circle caused the rope to become twisted, and the slightest twist of the rope was sufficient to rotate the centrifuge. At each such turn an electric contact was made and recorded on a very slowly moving kymograph by means of a signal magnet. In this manner a continuous 24-hour record of the dog's activity was obtained. It is obvious that the record gave *positive* information only. No recording by the signal magnet did not mean that the dog was necessarily asleep, although that was generally the case. Typical strips from such records are presented in

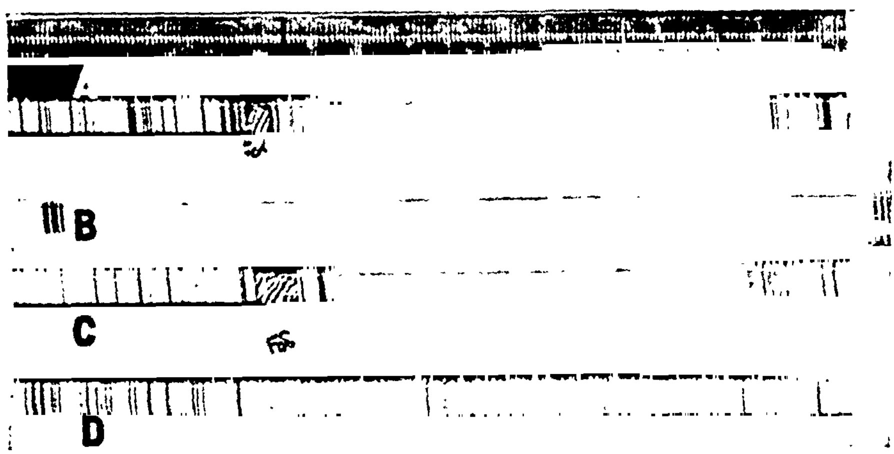

Fig. 1. Signal magnet records of the activity of decorticated dog A, each mark on the paper representing, as a rule, one circular walking movement. When the dog is running the marks coalesce forming a band. Time in minutes, total time of each record about three hours. Records A and C show the short lasting activity after feeding (indicated by oblique marks), followed by about one and a half hours of rest. Record B shows a rest period of three hours' duration, and record D, a period of activity lasting more than three hours.

figure 1. The 24-hour records showed that the decorticated dogs were active or at rest without any regard to day and night. On the average, the dogs spent from 10 to 45 minutes out of every hour in activity, the figures varying somewhat in the different dogs, or in the same dog on successive days. A slightly greater proportion of the time was spent in activity during the day than at night, but this was probably due to the disturbing noises of the laboratory during the working hours. The longest period of activity on record was 6 hours and 40 minutes of incessant walking. As a rule, there were about five or six periods of activity alternating with periods of rest and sleep each 24 hours.

Practically all experimenters state that hunger increases the activity of

decorticated dogs. None of them made any records of gastric contractions, and therefore no objective evidence was brought forward to show that the dogs were hungry. Of course, in a general way, one would expect dogs to be hungry if they had not been fed for some time. The activity records of our dogs show no definite increase at the time when the animals could be expected to be hungry. There was always the same haphazard alternation of activity and rest. Satiety, on the other hand, did result in a temporary decrease in activity. After they were fed the dogs usually walked around for a while, often urinated and defecated during that period, then lay down and slept for an hour or two. Figures obtained on dog A for seventeen days showed that after feeding he walked from 5 to 60 minutes (average, 29 minutes), then slept from 30 minutes to 6 hours (average, 2 hours 11 minutes).

Discussion. On the basis of our experience it is easy to distinguish sleep from wakefulness, as the two are seen in the normal human adult. During the waking state we are constantly analyzing the mass of impulses reaching the cerebral cortex from the sensory periphery and elaborating responses to the various stimuli, all in the light of our previous experience. This critical reactivity, as differentiated from stereotyped reflex responses, is lost during sleep. Very young animals or infants are incapable of critical reactivity (as determined by the usual tests), and we must therefore have a simpler criterion of sleep as it is seen in these creatures. One of the most satisfactory indications of sleep is the raising of the threshold of reflex excitability which accompanies its onset in the young as well as the old. Piéron (1913) stresses the value of this test in discussing the alternate states of rest and activity seen in some invertebrate marine animals, usually coinciding with such external changes as day and night, or ebb and tide. According to Piéron, the excitability of these animals does not decrease when they are quiescent, and he therefore refuses to call that state of inactivity sleep. For this reason we were particularly interested in testing the reflex excitability of our decorticated dogs at different times, when they were merely lying down and when they appeared to be asleep. The decrease in irritability which we observed indicates that decorticated dogs really sleep.

Up to this point we have simply confirmed the observations and conclusions of previous investigators, bringing out some additional facts to support their views. There still remains, however, to be answered the question whether the sleep of decorticated dogs differs in any respect from that of normal dogs. We have found a positive answer to this question in the fact that normal dogs can stay all up day and then sleep through the night, whereas decorticated dogs have half a dozen periods of sleep in 24 hours. The investigations of Szymanski (1918, 1920) demonstrated conclusively that some animals have one long period of rest in 24 hours, others, several such periods. He termed them monophasic and poly-

phasic animals. The polyphasic are the "lower" animals which make little use of their distance receptors for obtaining information about what is going on around them. Szymanski points out, however, that even among the monophasic animals, including man, the young are polyphasic.

Very young puppies, like infants, are blind and deaf, even though they possess good visual and auditory reflexes. They are typically polyphasic creatures in that they wake up every few hours, complain, are nursed, go to sleep again. Adult dogs are quite definitely monophasic, in spite of the fact that many of them will doze off at odd hours of the day, when there is nothing worth their attention going on, just as humans will under similar circumstances. This faculty of monophasic rest, or diurnal sleep, is permanently lost after decortication, which means that the dogs become once more polyphasic, reverting to the state of young puppies. Since puppies and new-born infants are, physiologically speaking, without a cortex, we are led to the inevitable conclusion that diurnal sleep depends for its development, and once developed for its maintenance, upon a physiologically active cerebral cortex. The behavior of an anencephalous child as reported by Edinger and Fischer (1913) well supports this view. One of the first things a baby learns is to sleep through the night, although it may still have two or more short naps during the day. The child in question died when it was nearly four years old, and yet it never learned to stay awake during the day, as it never learned to recognize its mother. Indeed, the authors compare the child to a new-born baby, "der praktisch auch ohne Grosshirn ist."

What bearing have our results upon the theories concerning the existence and localization of a sleep center? The existence of a subcortical sleep center is not at all a necessary postulate. In decorticated dogs and in new-born babies the state of wakefulness may be produced and maintained by the stream of afferent impulses from the surface receptors, proprioceptors, and the visceral receptors, especially the latter (hunger, thirst, distention of bladder and rectum). The diminution of this stream of impulses, when it reaches a certain critical value, results in a raising of the threshold of reflex irritability, producing sleep. However, our results are not in conflict with the eventual possible localization of a subcortical sleep center. The influence of such a center may be to keep an animal constantly asleep, unless there are enough afferent impulses coming in to inhibit the continuous action of that center; or the center may be in reality a waking center, whose activity will keep the animal awake, provided it is suitably reinforced by a sufficiently great number of afferent impulses. Philogenetically and ontogenetically, as distance receptors develop and play an important part in supplying the animal with information concerning the outside world, and as critical reactivity appears, the constant analysis of, and elaboration of responses to, the great variety of afferent impulses reaching

the cerebral cortex are sufficient to keep the animal awake for a long time, but not indefinitely. Sooner or later darkness will diminish the number of visual impulses, fatigue will lead to muscular relaxation and a decrease of proprioceptive impulses, and so on, until sleep is precipitated. We see that diurnal sleep, developed around the 24-hour cycle of day and night, is thus a definitely cortical phenomenon, has its center or is localized in the cerebral cortex, disappears when the cortex is removed. That explains why all those who study the sleep of higher animals and man cannot help but see the tremendous influence of cortical processes, habits, conditioned reflexes, etc., upon the onset of sleep. In man the habit of going to bed at certain hours, and of waking up at a definite time each morning, is not inborn, but acquired by experience. That the subcortical sleep tendencies are ever ready to exert their action is demonstrated by the fact that most people can fall asleep not only at their habitual bedtime, but almost at any time, if a condition is brought about where the number of afferent impulses reaching the cerebral cortex is greatly reduced. After a meal the primordial tendency to sleep is especially powerful and asserts itself with great ease, if permitted to do so.

To repeat, there is no conflict between the subcortical and the cortical theories of sleep. The first account for sleep in general, the second for the special diurnal type of sleep seen only in higher animals and in man.

SUMMARY

1. Decorticated dogs have several periods of sleep alternating with periods of activity each 24 hours.

2. Their activity consists of almost incessant walking in circles.

3. The most constantly occurring period of sleep follows very shortly upon feeding.

4. The conclusion is made that diurnal sleep in dogs depends upon the presence of the cerebral cortex for its establishment and persistence.

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THE SIGNIFICANCE OF FIBRE DIAMETER IN THE SENSORY NERVES OF THE CRUSTACEAN LIMB

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Contributions from the Bermuda Biological Station for Research and the Osborn Zoological Laboratory, Yale University

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In recent years a number of important papers have appeared concerning the functional significance of nerve fibre diameter (cf. Lapicque, 1926, Gasser and Erlanger, 1927) but as the writer has pointed out (Barnes, 1930) more extensive observations are needed before any generalizations can be drawn. For studies of nerve fibre diameter a favorable preparation may be found in the crustacean limb. De Renyi (1929) in particular has published careful measurements of the variation of fibre diameter in the crustacean nervous system, in which the diameter of nervous elements may vary from 2 to 125μ.

Previous investigators have overlooked the fact that Biedermann (1895) was the first to suggest that the wide variation in the diameter of nerve fibres must have profound physiological significance. He states "One important fact that has hitherto been overlooked is the marked variation in calibre which occurs in both medullated and non-medullated central and peripheral nerve fibers. If, as we might expect, this is related with functional dissimilarity the mere anatomical differences (apart from physiological reasons to be considered below) would be decidedly against the homogeneity of all nerve-fibres so often insisted on according to which the differences of excitatory effect must be referred solely to differences in the terminal organs."

With the exception of Biedermann's statement all previous communications concerning the physiological significance of fibre diameter have been reviewed by Lapicque (1926) and for this reason it seems unnecessary to present a complete historical survey in the present paper. The first actual observations were reported by Lapicque and Legendre (1913) who correlated the chronaxie of various fibres in the frog with their diameter. The specific character of nerve fibres was not known to Langley for in his important paper on peripheral nerves (1922) he puts forward the suggestion that the diameter of the fibres is determined by the organ to which they run. The original observations of Lapicque were later confirmed by Lapicque and Desoille (1927) and Kreindler (1927) with a number of minor corrections, for example, the diameter of the large fibres, 20μ, becomes 16–18μ.

Erlanger and Gasser (1924) found that a single electrical stimulus evokes a series of waves travelling at different speeds; this phenomenon, however, was not found in the phrenic nerve whose fibres are of uniform size. Gasser adopted Lapicque's notions concerning the functional significance of fibre diameter (cf. Lapicque, Gasser and Desoille, 1925) and in subsequent papers written with Erlanger (1927, etc.) presented evidence indicating that the large or A waves are mediated by large motor and proprioceptive fibres, the medium waves are concerned with touch and temperature, and the small fibres convey pain impulses. The hypothesis that pain is in some way associated with activity in nerve fibres of small diameter has received additional support (cf. Gerard's review, 1930; Adrian, Cattell, and Hoagland, 1931). The slow waves which Adrian elicited (1930) by the application of weak acid to the skin may be set up by crushing or burning. These impulses may help to throw light on the C wave of Gasser. However, it must not be forgotten that rapid fibres may sometimes be involved with pain sensations (Adrian, 1930).

The paper of Matthews (1929) may be consulted for measurements of fibres in frog nerve associated with "specific" proprioceptive and cutaneous impulses.

Other aspects of the nerve fibre diameter question have been considered by Gothlin (1908, 1917), Thöle (1912) and Kiss and Mihalik (1928). Umrath (1928) found that this relationship does not apply to excitatory processes in plants.

The present paper deals with results secured at the Biological Station in Bermuda during the summer of 1931. The object of the investigation was to measure the diameter of living sensory nerve fibres and if possible to establish a relationship between the rapidity of movements in the crustacean limb and the cross section of sensory fibres running to the receptive fields involved in the reflex arcs. Over two thousand living sensory nerve fibres were measured in sea water and their receptive fields determined by tactile stimulation of areas on the limb extremities. The time relations of the opening and closing reflexes of the claw and the extension and flexion of the walking limbs were measured with a stop watch. The receptive fields were mapped out by stimulation of the exoskeleton by means of a fine bristle. As is well known, a contact stimulus applied to the inner or biting edges of the claw produces closing and the same stimulation at other areas of the claw elicits opening.

In *Goniopsis cruentatis* there is a restricted linear area immediately above the biting edge of the claw teeth which is unusually sensitive and a single tactile stimulation of this sensitive line elicits immediate opening of the claw. The soft interior of the claw was gently drawn out without disturbing the position of the nerve fibres. The portion supplying the receptive field for opening of the claw was cut away from the rest of the prepara-

tion and examined microscopically in sea water. The nerve strands were teased apart with fine glass needles or with an Emerson micromanipulator.

In all preparations it was found that the individual sensory fibres innervating the regions of the shell stimulated varied from 2 to 20μ in diameter but the nerve strands which were involved in the more rapid reflex (closing of the claw) contained a higher proportion of fibres of large diameter

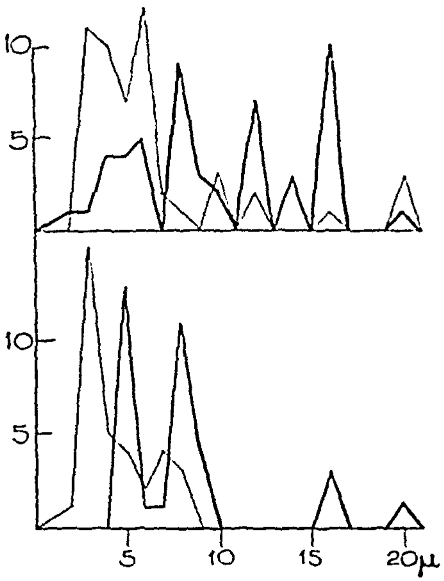

Fig. 1

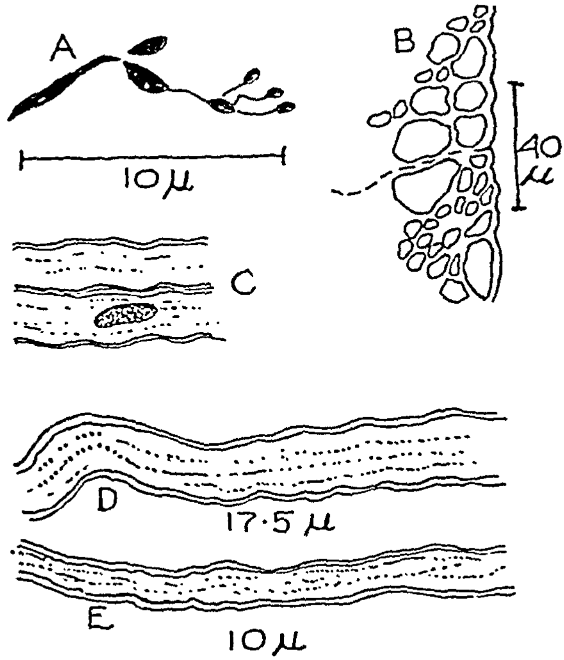

Fig. 2

Fig. 1. The distribution of the diameters of sensory fibres in the claw of *Goniopsis*. Abcissae: diameters in microns. Ordinates: number of fibres in each diameter group. Upper graph: fibres in the fixed digit; lower graph: fibres in dactylus (motile digit). The heavy line in each represents sensory fibres innervating the receptive field of the biting edges of the claw. Stimulation of this region elicits rapid closing of the claw. The light lihe represents the fibres of smaller diameter innervating the remaining parts of the claw surface. Stimulation of these regions produces slow opening of the claw. See table 1 for the time relations of the reflexes of this claw prior to excision.

Fig. 2. Nerve fibres in crustacean limb. A. The Retzius artefact. These curious "nerve endings" appear in preparations of the contents of the dactylus when stained with methylene blue. Vom Rath (1890) showed that gold chloride reveals additional sensory cells.

B. Cross section of the meropodite nerve in the walking leg. Note the wide variation in fibre diameter.

C. Two sensory fibres; vital staining with methylene blue; note the large oval nucleus and the close proximity of the two fibres.

D. A 17.5μ sensory fibre innervating the biting edge of the claw.

E. A 10μ sensory fibre innervating the receptive field for the opening of the claw.

(fig. 1[1]). The arrangement of the diameter groups is more heterogeneous than the tactile sensory fibres in large specimens of *Cancer pagurus* mentioned in a preliminary note (Barnes, 1931) and resembles more closely the innervation in the limbs of several small Mediterranean crabs (Barnes, 1930).

The rather striking difference in the rapidity of the two reflexes of opening and closing the claw may be seen in table 1 which records observations made on the animal whose sensory fibres are represented in figure 1. The closing reflex involving the large sensory fibres is frequently four times as rapid as the opening of the claw and the latter reflex often requires repeated tactile stimulations for maximum movement. It is obvious that the large sensory fibres play an essential rôle in the more rapid movements of the claw. In vertebrate nerve it has been found (Erlanger, 1927) that fibres

TABLE 1*

Slow and rapid reflexes in the claw of Goniopsis

REFLEX	EXTENT OF MOVEMENT	REGION STIMULATED	NUMBER OF STIMULATIONS	TIME FOR COMPLETED MOVEMENT
	mm.			*seconds*
Opening	5	Dorsal surface of dactylus	4	3.2
	3	Sensitive line below teeth of fixed digit	3	1.6
Closing	5	Teeth of fixed digit	1	0.2
	5	Teeth of dactylus	1	0.8

* See figure 1 for sensory fibre diameter in this animal. Similar results were secured with over one hundred other animals.

of 18μ diameter are twice as rapid as fibres of 9μ diameter and three times as rapid as 6μ fibres. It has been suggested (cf. Gerard, 1931) that the more rapid conduction in the thick fibres of vertebrate nerve may be correlated with the greater distance between the nodes of Ranvier. In the unmedullated nerves of crustacea, however, the significance of fibre diameter is not obscured by complicating factors introduced by the structural variations of the fibre sheath.

A cross section of the nerve (fig. 2, B) confirms the variation in fibre diameter observed in the living preparations (fig. 2, C, D, E). Unfortunately the histological evidence is not yet supported by records of the action currents in the various fibres. Through the kindness of Prof. E, D. Adrian the writer was able to make several oscillograph records showing

[1] The figure is selected from many others representing approximately the same distribution of diameter groups.

the discharges of sensory impulses in the limb nerve, elicited by tactile stimulation of the ventral surface of the dactylus[2] and by bending the joints of the limb (fig. 3). It is interesting to note that these impulses are clearly larger than the "spontaneous" sensory discharges occurring in the same preparations which are probably arising in chemoreceptors in the skin stimulated by evaporating sea water. It is suggested that the impulses produced by chemical stimulation are conveyed by nerve fibres of small size. The graphs showing the distribution of the diameter groups indicate a large proportion of small fibres (fig. 1) and it is not improbable that some of these innervate chemoreceptors in the skin. Luther (1931) has shown that the extremities of the crustacean limb are the principal receptive fields for the chemical sense.

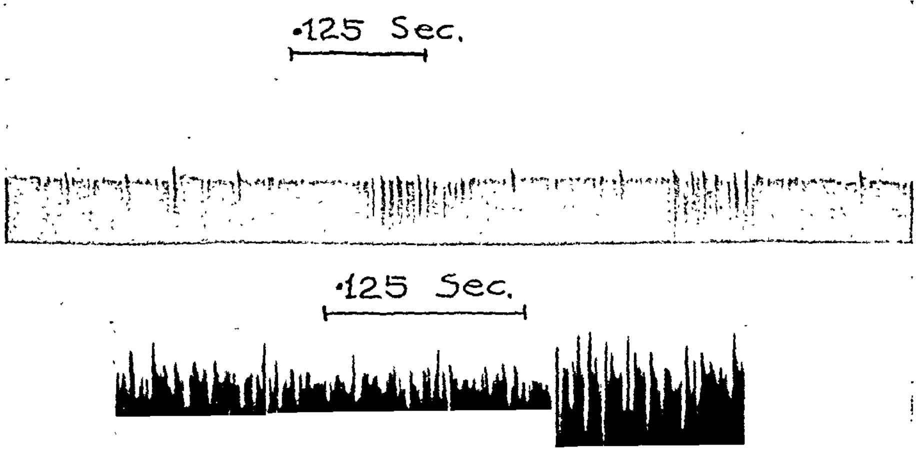

Fig. 3. Oscillograph records of sensory discharges in the crab's limb. Upper record: two short discharges of large tactile impulses produced by bending the ventral hairs of the dactylus. These are superimposed on smaller impulses occurring persistently at low and irregular frequencies, probably from chemoreceptors, stimulated by evaporating sea water. Lower record (faster film and higher magnification): "Spontaneous" persistent impulses as above and at right a discharge of larger impulses from movement receptors in the joint produced by bending the limb.

In the present investigation only the sensory fibres were examined. The unique mechanism of peripheral inhibition complicates the study of motor fibres which will be the subject of further work.

In conclusion it may be said that the histological measurements supported by observations of the receptive fields for tactile stimulation and their associated reflexes indicate that the diameter of sensory nerve fibres has important physiological significance. Those movements which are executed rapidly by the animal involved reflex arcs whose loci for stimula-

[2] The dactylus contains many sense cells. When vitally stained with methylene blue these resemble the "nerve endings" of Retzius (see fig. 2, A).

tion are innervated by sensory fibres of large diameter. It is clear the problem of nerve fibre diameter must remain in an unsatisfactory state until the position of the large and rapid fibres is determined with reference to the movements of the organism as a whole. It will be recalled that the large sensory fibres in vertebrates (proprioceptive) are those involved with reflexes which are constantly in action (maintaining posture).

SUMMARY

The rapid closing movement of the crustacean claw involves sensory fibres of larger diameter than those innervating the receptive fields for the slow opening reflex.

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STUDIES AT HIGH ALTITUDE[1]

Blood Observations on the Indian Natives of the Peruvian Andes

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The mental well-being and the capacity for physical work are far superior and nearer normal in the native of the altitude, as compared with a man born at sea level and who lives accidentally under such altered condition of a low barometric pressure. It is logical to assume that the optimal physiological processes, which make possible for the organism to thrive favourably under a low oxygen tension, will be found in the study of the man whose adaptation represents a process of centuries. Then perhaps we will interpret, as it must be logically so, the failure of adaptation from the point of view of deviation from the physiology of the native of the altitude, and not from the physiology of sea level, as we do it today.

A number of observations on certain of the blood characteristics of the Indian natives of the Peruvian Andes will be presented in this paper. The investigation was conducted in the town of Morococha, Peru, a mining town at an altitude of 14,890 feet, and with an average barometric pressure of 410 mm. Hg. Temperature variations in this place range from 0° to 30°C. (23°C. being the average inside temperature in the laboratory). A few humidity observations made during the summer season gave values between 37 and 77 for relative humidity. The subjects studied have been carefully selected as normal men (not exposed to the dusty inhalation in the mines), from 18 to 76 years of age, with 88 per cent between 18 and 49 years and with a general age average of 30 years. These men were natives of this town or born in neighboring places of about 10,000 feet elevation, and with a residence of at least a year in Morococha. In most cases the Indians examined had never been at sea level.

Red cell count determinations. A number of observations have been made on the changes in the red cell count brought about by exposure to high altitudes. Practically all investigators agree in the statement that a polyglobulia (or erythrocytosis) is found in most residents of the altitude, and

[1] These investigations have been partly supported by the Medical Faculty of the University of San Marcos (Lima, Perú).

that it is also produced in individuals and animals shortly after being exposed to the low oxygen tension.

We have determined the red cell count on 132 normal male natives described above. Bureau of Standard pipettes and double Levy chambers were used, and in every case two counts were made taking the average as

TABLE 1

Summary of the blood findings

NUMBER OF OBSERVATIONS	DETERMINATION	MEAN	STANDARD DEVIATION	COEFFICIENT VARIATION	VARIATIONS
				per cent	
132	No red cells per 10^6	6.66±0.06	1.00±0.04	15.0	4.80 to 10.40
100	Gms. Hgb. per 100 cc.	15.93±0.09	1.39±0.07	8.7	13.4 to 18.9
100	Corpuscular Hgb. $\gamma\gamma$	24.4±0.14	2.7±0.13	11.6	18 to 31
25	Hematocrit (R.B.C. %)	71.1±1.34	7.6±0.73	10.8	57 to 86
25	Corpuscular volume. Cu. micr.	96.2±1.49	11.0±0.95	12.8	70 to 121
25	Corpuscular Hgb. concentration %	24.9±0.33	2.2±0.09	9.0	20 to 29
113	Blood viscosity	8.6±0.14	2.2±0.10	25.5	5.2 to 15.2
95	Blood coagulation time. Minutes	6.5±0.12	1.7±0.05	26.3	3.5 to 13
120	Leucocyte count Per cu. mm.	5,210±84.5	1,370±59.7	26.3	2,300 to 9,100

		AVERAGE	VARIATIONS
7	Serum viscosity	1.95	1.9 to 2.0
9	Plasma viscosity	1.80	1.7 to 1.9
14	Fragility of red cells		
	Minimal hemolysis	0.30	0.50 to 0.20
	Maximal hemolysis	0.22	0.38 to 0.12
12	Van den Bergh reaction	+in 91%	
8	Plasma bilirubin, mgm.	0.7	0.2 to 1.1
53	Leucocyte count		
	Polynuclears %	69.0	36 to 90
	Lymphocytes	24.5	4 to 44
	Eosinophiles	0.3	0 to 2
	Monocytes	6.1	0 to 24
	Premyelocytes	+ in 28% of the cases	
	Histiocytes	+ in 40% of the cases	

the final result. Table 1 presents the results obtained. The mean value is 6.66 ±0.058 millions per cubic millimeter, which is a little more than a million higher than the average normal value at sea level (the series of observations made in this country by Osgood (1) in 1926; Wintrobe and Miller (2) in 1929, and most recently by Foster and Jhonson (3), who have

studied 352 normal male adults represent the most accurate basis for normal blood values at sea level. The average normal red cell count from these series is 5.50 millions per cubic millimeter). But we have to pay attention to a very interesting fact, and this is that 26 per cent of the people examined showed a red cell count below six millions, and about 11 per cent of them definitely lower than the normal average at sea level. So there is a considerable percentage of these natives who live at an altitude of almost 15,000 feet with a red cell count which would be considered normal at sea level. Necessarily we come to the important conclusion that although an increased red cell count is the most common finding at high altitudes, a normal sea level count is not incompatible with health in these high regions.

The extreme values found in the red cell count determinations, from 4.80 to 10.40 millions per cubic millimeter, indicate marked individual variations. The coefficient of variation is 15 per cent and the standard deviation 1 million which, compared with the 0.37 million obtained in the series of Foster and Jhonson (3), shows a higher variation in the erythrocyte count at high altitudes as compared with sea level.

There was no relationship between the red cell count and age. The average of the erythrocyte count was practically the same in young and old individuals. There was no relation either between the magnitude of the cell count and the bodily characteristics of the people examined.

Hemoglobin determinations. Numerous investigations on the hemoglobin content of the peripheral blood appear to indicate that an increase of this substance is one of the compensatory processes to the low oxygen tension.

Table 1 shows the results obtained in 100 determinations made on normal Indian natives. These determinations were made with the Sahli hemoglobinometer and the Newcomer colorimetric procedure, both standardized several times with the oxygen combining power method of Van Slyke and Stadie (4). Most of the observations were made in the morning and with the subject in a resting condition. We see that the mean value of these 100 determinations is 15.93 $\pm$0.09 grams of hemoglobin per 100 cc. of blood, a value very slightly higher than the normal average at sea level (which is 15.75 grams according to the series of observations already mentioned). For practical purposes we may consider both values as being identical. In our series the median value is equal to the mean indicating that exactly half of the people examined have a higher value, and the other half a lower value, than the normal average at sea level. The variations range from 13.4 to 18.9 grams per 100 cc. of blood, a range which is almost equal to the one found at sea level (from 13.2 to 19.0 grams).

These results immediately suggest the question: Is an increase in the hemoglobin of the circulating blood not an essential compensatory process

for adaptation at high altitudes? It has been assumed that the increase of hemoglobin functions to increase the oxygen content of the circulating blood, thus supplying oxygen to the tissues without a great diminution in its tension. But late evidence appears to indicate that such a change is not as important as it has been considered. Summervell (5) found some natives at a high altitude with a low percentage of hemoglobin were far more efficient that European members of expeditions with a higher percentage. Cohnheim and Krelinger (6), and Gross and Kestner (7) reported a fall of hemoglobin at a high altitude, following strenuous physical activity, and although this occurrence may be explained by increased hemolysis, the fact remains that they were able to support the strain with a lower percentage of hemoglobin. Campbell (8), (9) considers the increase in this substance merely as a symptom, and the recent investigation of Dill, Edwards, Föllig, Obert, Pappenheimer and Talbot

TABLE 2

Gaseous determinations in arterial blood (Oroya—12,200 feet)

NON-RESIDENTS	CO_2	O_2 CONTENT	O_2 CAPACITY	SATURATION
	vols. per cent	*vols. per cent*	*vols. per cent*	*per cent*
1	32.1	19.5	21.7	88.5
2	31.2	21.1	22.1	94.0
3	32.7	18.9	19.9	93.3
4	31.0	20.2	21.6	92.1
5	32.3	20.8	22.4	91.6
NATIVES				
1	34.2	17.3	20.0	84.5
2	30.5	16.5	19.2	84.1

(10) indicates no relation whatsoever between the degree of this increase and the symptoms of oxygen want. The Cerro de Pasco Expedition (11) concluded that the Indian natives of the Peruvian Andes have a lower saturation compared with the white residents and members of the expedition. These observations were later confirmed by our studies (12) of the same natives at Oroya (12,200 feet elevation). The results obtained in our determinations are presented in table 2. We see here that a group of individuals, although of Indian race too, but who had previously been at sea level for quite a prolonged time, have an average arterial saturation of 91.9 per cent as compared with 84.3 per cent obtained in two natives of the locality and who represent a maximum adaptation. Both the content and the oxygen capacity were lower in the individuals adapted to altitude.

The low saturation of the arterial blood in these natives has also been demonstrated in our studies at Morococha. In three people examined the

average oxygen saturation was 83.5 per cent, a result which is in very close agreement with the ones obtained at Cerro de Pasco and Oroya. If we know that the native lives at high altitudes with a lower saturation of his arterial blood, and with less circulating oxygen, as compared with the recent arrival, and yet is much more physically efficient than the latter, we have great difficulty in accepting the assumption that an increase of hemoglobin is of great importance.

The relationship between the red cell count and the hemoglobin is interesting and important. We have calculated the "mean corpuscular hemoglobin" (amount of hemoglobin in each red cell expressed in micro-micro grams $\gamma\gamma$) in the 100 people examined. The mean value of the corpuscular hemoglobin in these cases is 24.4 ±0.183, which corresponds

TABLE 3

Erythrocyte counts and corresponding averages

NUMBER OF RED CELLS PER 10^5	AVERAGES				
	Hgb. per 100 cc.	Corpuscular Hgb., $\gamma\gamma$	Hematocrit RBC	Corpuscular volume	Corpuscular Hgb. conc.
	grams		*per cent*	*cu. microns*	*per cent*
4.50–4.99	14.8	30.0			
5.00	14.7	27.4	65.0	119	25.0
5.50	14.6	25.0			
6.00	15.6	24.6	66.6	102	25.0
6.50	16.5	24.0			
7.00	16.3	22.0	68.3	93	25.2
7.50	16.1	20.6			
8.00	17.1	20.5	74.6	89	23.8
8.50	17.1	19.3			
9.00	17.9	18.5	76.6	82	23.5

to a color index of 0.84 taking 5.00 million red cells per cubic millimeter and 14.5 grams of hemoglobin per 100 cc. of blood as 100 per cent. Taking 29$\gamma\gamma$ as the average normal value at sea level, it appears that in these natives each red cell contains less hemoglobin (15 per cent reduction). We will return later to the interpretation of this finding. The standard deviation of the corpuscular hemoglobin in our series is 2.7 ±0.129$\gamma\gamma$ compared with 1.68 ±0.08 at sea level, showing a higher range of variation at high altitude.

Normally at sea level there is a close correlation between the red cell count and the amount of hemoglobin contained in each corpuscle. This correlation is inverse, that is, the higher the red cell count the lower the corpuscular hemoglobin. This correlation is also demonstrated at high altitude as we see in table 3. As the red cell count increases the corpuscular

hemoblogin becomes consistently less. The correlation coefficient between the erythrocyte count and the corpuscular hemoglobin is -0.7987 ± 0.067, a value even higher than that found at sea level (the correlation coefficient between these two characteristics calculated on the determinations of Osgood and Wintrobe and Miller's normal series is -0.6258 ± 0.043).

From the corresponding regression equations[2] the diagram of figure 1 has been constructed, and shows in a graphic form the corresponding values at

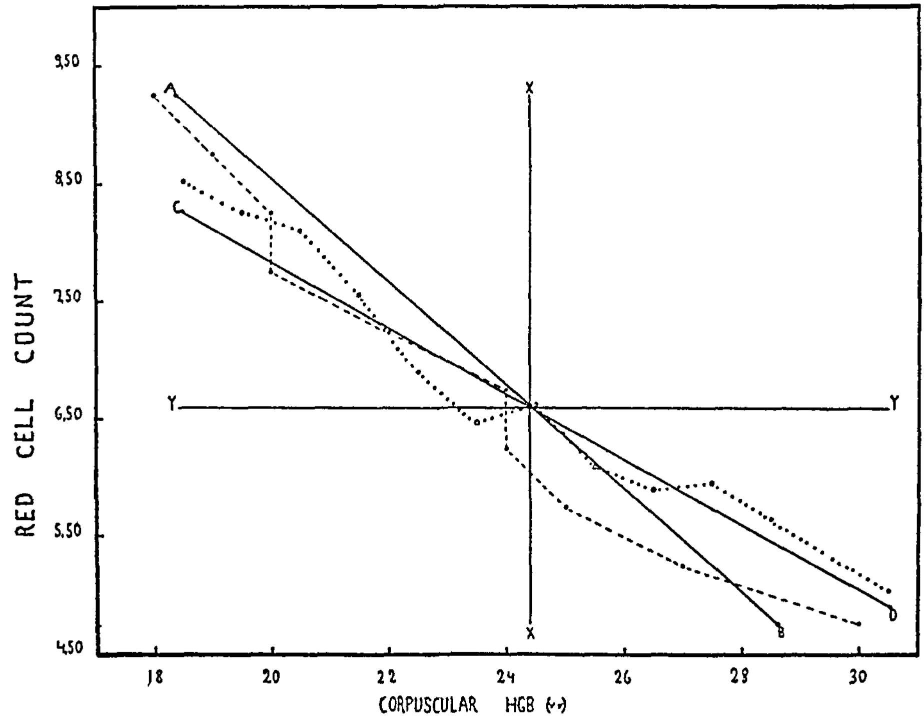

Fig. 1. Observed and calculated regressions for corpuscular hemoglobin and red cell count at high altitude. Lines (----------) are the means of the observed corpuscular Hb arrays. *AB* is the corresponding regression line of corpuscular Hb on red cell count. Dots (........) are the means of the observed red cell counts on corpuscular Hb. *CD* is the calculated regression line of red cell count on corpuscular Hb. *XX* gives the location on the corpuscular Hb scale of the mean of all 100 determinations. *YY* gives the mean of the red cell count of the 100 determinations.

any level of the red cell count, at any value of cell hemoglobin, in these normal natives of high altitude.

That the variations in corpuscular hemoglobin do not depend on the amount of hemoglobin per 100 cc. blood is shown by the correlation coefficient between these two characteristics, which is only 0.0231 ± 0.067. The correlation ratio is also valueless.

[2] The corresponding regression equations are:

$$\text{Corpuscular hgb.} = (-2.274 \times \text{red cell count}) + 39.43$$
$$\text{Red cell count} = (-0.28 \times \text{corpuscular hgb.}) + 13.44$$

The close correlation between the red cell count and the cell hemoglobin which is found normally at sea level and at high altitudes must be far from an accidental finding. It is quite possible that it is related with the proper supply of the blood oxygen to the tissues. That this correlation is more perfect at high altitudes, where the low oxygen tension is a handicap for this process, is quite significant and important.

In table 4 we present the results obtained in gaseous determinations of six samples of blood, three corresponding to arterial and three to venous blood. The technique used in these determinations was that of Van Slyke and Stadie (4). In our three arterial determinations the average oxygen content was 21.4 volumes per cent, in close agreement with the normal sea level value of 21.0. The average oxygen capacity of 25.6 volumes per cent in the arterial blood is distinctly higher than at sea level, while in the venous blood 22.8 is in closer agreement. The increased oxygen combining power of the blood at high altitude may appear at first sight as indicating an increased amount of hemoglobin in each corpuscle, but we have calculated the ratio: $\frac{O_2 \text{ vol. capacity per 1000 cc. blood}}{\text{Mill. red cells per cu. mm.}}$ which will give the amount of

TABLE 4

Gaseous determinations in arterial and venous blood

	NUMBER OF RED CELLS PER 10^6	CO_2 CONTENT	O_2 CAPACITY	O_2 CONTENT	CORPUSCULAR HGB., $\gamma\gamma$	RATIO: $\frac{O_2 \text{ CAPACITY}}{\text{RED CELLS } 10^6}$	RATIO: $\frac{O_2 \text{ CONTENT}}{\text{RED CELLS } 10^6}$	COLOR: COUNT RATIO	OXYGEN SATURATION
Arterial blood									
		vols. per cent	*vols. per cent*	*vols. per cent*					*per cent*
1	6.74	28.9	25.1	21.2	27.7	37.2	31.4	0.96	82.9
2	7.14	30.9	24.8	20.7	25.9	34.7	28.9	0.90	81.2
3	8.74	30.9	27.0	23.4	23.0	30.8	26.7	0.80	85.2
Average...	7.54	30.0	25.6	21.4	25.5	34.2	29.0	0.88	83.4
Venous blood									
1	5.87		22.4		28.4	38.1		0.99	
2	6.67		22.8		25.4	34.1		0.88	
3	8.17		23.4		21.3	28.6		0.73	
Average...	6.90		22.8		25.0	33.6		0.86	
Normal at sea level									
	5.50		21.1	21.0	28.8	38.3	38.1	1.00	95.0

oxygen that each cell may take on exactly the same scale of value as the corpuscular hemoglobin. We see that the average of this ratio in the arterial blood is 34.2 and 33.6 in the venous blood, or 10.7 per cent and 12.2 per cent lower than the ratio at sea level which is 38.3.

The lesser oxygen combining capacity per red cell is a further and final proof of the lesser amount of hemoglobin in each red corpuscle, as indicated in our direct hemoglobin determinations. The corpuscular hemoglobin calculated on the basis of these oxygen determinations falls below the normal average at sea level. Table 4 also shows the inverse relation between the red cell count and the cell oxygen capacity, both in the arterial and in the venous blood, that is, the higher the count the less oxygen capacity, analogous to the relationship between the erythrocyte count and cell hemoglobin. The ratio: $\frac{O_2 \text{ vol. content per 1000 cc. blood}}{\text{Mill. red cells per cu. mm.}}$ is definitely lower in the arterial blood at high altitude as compared with sea level (29.0 and 38.1 respectively). Thus the red cell of these natives comes to the tissues holding less oxygen in its hemoglobin. This is a very important point to take in consideration.

The color:count ratio calculated by the formula of Peters and Van Slyke (13) also gives values lower than 1, corroborating again the diminished hemoglobin content in each corpuscle. It shows the same striking inverse relation to the level of the red cell count.

Red cell volume determinations. Determinations have been made in 25 normal males with the Van Allen hematocrit tube. In every case two hematocrit tubes were filled, and a red cell count and hemoglobin determination made simultaneously on the same sample of blood. The tubes were centrifugalized for over an hour in an electric centrifuge, and the average of the two determinations was taken as the final result. The hematocrit determinations made at high altitude are distinctly higher than those found at sea level. The mean value of these determinations is 71.1 $\pm$1.34 red cell percentage and the variations range from 57 to 86 per cent. The mean value of the corpuscular volume in this series is 96.2 $\pm$1.49 cubic microns, which compared with the normal average of 82.2 at sea level means an increase of 16 per cent.

Some of our hematocrit determinations give unusual high figures which might be due to incomplete packing of the red cells on account of their large volume and the small bore of the hematocrit tube. In order to check the cell volume determinations we have measured directly the diameter of the red cells in dry stained smears obtained in 20 other normal unselected individuals. A minimum of 100 cells has been measured in each smear by means of a calibrated micrometer. The average result of these measurements was 8.04 microns with variations between 7.78 and 8.52 microns. Accepting Haden's (14) investigation that a corpuscular volume of 92

cubic microns is equivalent to 7.7 microns diameter, we find that 8.04 bears a close agreement to the mean value of 96.2 cubic microns obtained in the volume determinations.

We have enough evidence from these observations to state that the erythrocyte of the peripheral blood in these natives has a higher volume when compared with that of sea level.

It has been demonstrated that each red cell contains less hemoglobin, and therefore with the present evidence of a larger volume it follows that the corpuscular hemoglobin concentration must be low. That this is true is shown in table 1. The cell hemoglobin concentration has been calculated by dividing the corpuscular hemoglobin by the corpuscular volume and multiplying by 100. The mean value of the cell hemoglobin concentration is 24.9 ±0.732 per cent as compared with 34.2 per cent, the normal average at sea level. It is extremely interesting that this low concentration is jealously maintained at high altitude (as it occurs correspondingly at sea level), as shown by the standard deviation which is only 2.2 ±0.09, and the coefficient of variation which is 9 per cent, demonstrating the tendency for keeping always about one-fourth of the red cell saturated with hemoglobin, independently of the level of the erythrocyte count.

In order to maintain this same concentration there must be a close correlation between the red cell count and the size of the red cell, as was the case with the corpuscular hemoglobin. In table 3 we find this correlation strikingly demonstrated. It shows that with an increasing count the corpuscular volume becomes consistently less, in a manner proportional and analogous to the decrease in the cell hemoglobin, with the result that the concentration is kept about the same.

On the basis of the different blood findings we have calculated several correlation coefficients and ratios which are given in table 5. The correlation between the red cell count and the corpuscular volume is quite definite. The correlation is linear and the coefficient is −0.6015 ±0.1348 which means that the size of the red corpuscle varies inversely with the cell number. With a high count the size of the erythrocyte tends to approach the normal sea level value. From the correlation coefficient the corresponding regression equations[3] have been calculated and represented in a graphic form in figure 2. The correlation between the hematocrit, or red cell percentage to plasma, and the corpuscular hemoglobin concentration is significant. The correlation is strictly linear and the coefficient of −0.8954 ±0.1348 denotes an almost perfect relationship between the two. A high cell proportion to plasma means invariably a lower corpuscular

[3] The regression equations are:

Corpuscular volume = (−6.16 × red cell count) + 142.7

Red cell count = (−0.058 × corpuscular volume) + 13.12

TABLE 5

Correlation between the different blood findings

CHARACTERS CORRELATED	ZERO-ORDER CORRELATION COEFFICIENTS AND RATIOS				
	Correlation coefficient	Correlation ratio	$\rho\ n^2 - r^2$	Corrected correlation ratio	$\sqrt{\frac{K-1}{N}} \pm 0.67449 \frac{1}{\sqrt{N}}$
Red c. count—Gms. Hgb.	+0.5507 ±0.0674	0.6174	0.0786 ±0.0340	0.5789	0.3000 ±0.0674
Red c. count—Corp. Hgb.	−0.7987 ±0.0674	0.8754	0.1284 ±0.0463	0.8652	0.3000 ±0.0674
Gms. Hgb.—Corp. Hgb.	+0.0231 ±0.0674	0.2764	0.0758 ±0.0349	0.2184	0.2236 ±0.0674
Red c. count—Hematocrit	+0.5475 ±0.1348	0.5313		0.4687	0.4000 ±0.1348
Red c. count—Corp. volume	−0.6015 ±0.1348	0.7427	0.1898 ±0.1045	0.7159	0.4000 ±0.1348
Red c. count—Corp. Hgb. conc.	−0.2655 ±0.1348	0.2602		0.1187	0.4000 ±0.1348
Hematocrit—Gms. Hgb.	+0.3675 ±0.1348	0.4776	0.0930 ±0.0756	0.2848	0.4900 ±0.1348
Hematocrit—Corp. Hgb.	−0.5440 ±0.1348	0.7206	0.2233 ±0.1116	0.6539	0.4900 ±0.1348
Hematocrit—Corp. volume	+0.3548 ±0.1348	0.6396	0.2832 ±0.1096	0.5444	0.4900 ±0.1348
Hematocrit—Corp. Hgb. conc.	−0.8954 ±0.1348	0.7848		0.7366	0.4900 ±0.1348
Gms. Hgb.—Corp. volume	−0.4591 ±0.1348	0.5804	0.1261 ±0.0869	0.4962	0.4472 ±0.1348
Gms. Hgb.—Corp. Hgb. conc.	−0.0311 ±0.1348	0.2730	0.0736 ±0.0677	0.0000	0.4472 ±0.1348
Corp. volume—Corp. Hgb.	+0.5024 ±0.1348	0.6230	0.1357 ±0.0986	0.5519	0.4472 ±0.1348
Corp. Hgb. conc.—Corp. volume	−0.4855 ±0.1348	0.8343	0.4603 ±0.0929	0.8090	0.4472 ±0.1348
Corp. Hgb. conc.—Corp. Hgb.	+0.4367 ±0.1348	0.5154	0.0749 ±0.0693	0.4066	0.4472 ±0.1348
Red c. count-Viscosity	+0.4140 ±0.0640	0.5977	0.1833 ±0.0472	0.5529	0.2846 ±0.0640
Viscosity—Corpusc. Hgb.	−0.2137 ±0.0695	0.3619	0:0853 ±0.0372	0.3038	0.2525 ±0.0695
Coagul. time—Red c. count	−0.1582 ±0.0692	0.3628	0.1066 ±0.0404	0.2502	0.3075 ±0.0692
Coagul. time—Viscosity	−0.2174 ±0.0687	0.3864	0.1021 ±0.0392	0.2894	0.2473 ±0.0687

hemoglobin concentration. The narrow range of variation in the latter is strictly related to the hematocrit figure. This correlation has such a high value that we have investigated whether it also exists normally at sea level. The result of this investigation (carried out on the figures given in the normal series of Osgood and Wintrobe and Miller) showed that this relationship is also evident, though in a much less degree, in normal people at sea level. The correlation coefficient is -0.5059 ± 0.048, that is, lower than at high altitude.

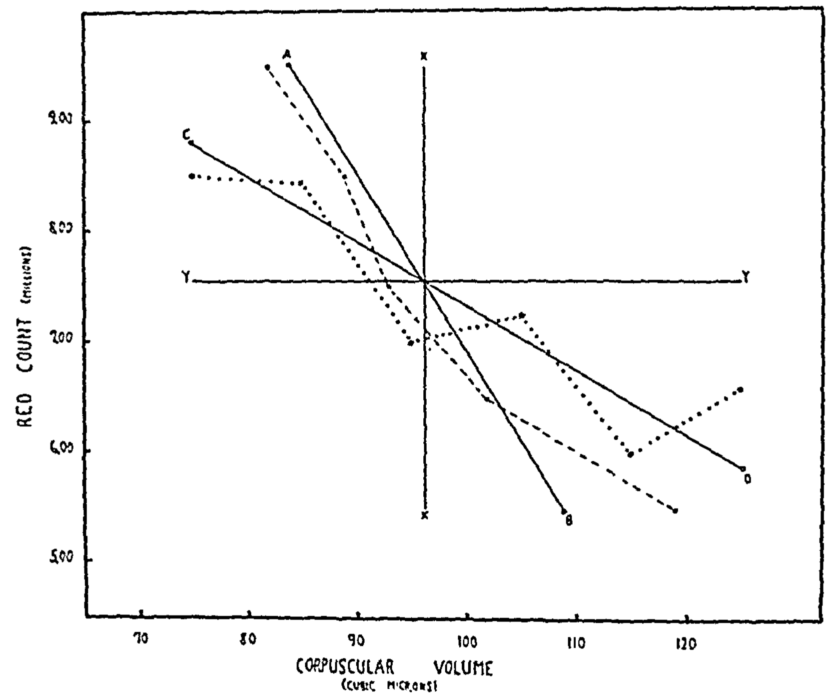

Fig. 2. Observed and calculated regressions for corpuscular volume and red cell count. Lines (----------) are the means of the observed corpuscular volume arrays. *AB* is the corresponding regression line of corpuscular volume on red cell count. Dots (........) are the means of the observed red cell count arrays. *CD* is the calculated regression line of red cell count on corpuscular volume. *XX* is the mean of the corpuscular volume in all 25 determinations. *YY* is the mean of the red cell count in the 25 determinations.

To demonstrate graphically this correlation, both at sea level and at high altitude, the corresponding regression equations have been calculated. The diagram of figure 3 shows that the line of correlation between the hematocrit and the cell hemoglobin concentration is almost identical and parallel in both cases, with the only difference that at high altitude it is placed at a higher level of red cell percentage and at a lower hemoglobin concentration.

From the above observations the erythrocyte of the circulating blood

may be represented schematically as in figure 4, that is, a bigger cell and with a diminished amount of hemoglobin and oxygen content. Such an arrangement of a larger surface area for the hemoglobin, and consequently for the oxygen, will undoubtedly favor the acquisition of oxygen and its unloading at tissue level, which is the fundamental and basic problem of altitude physiology.

If the hemoglobin is superficially distributed on the surface of the red cell, as it has been affirmed by Brinkman and Szent-Gzörgi (15) and by

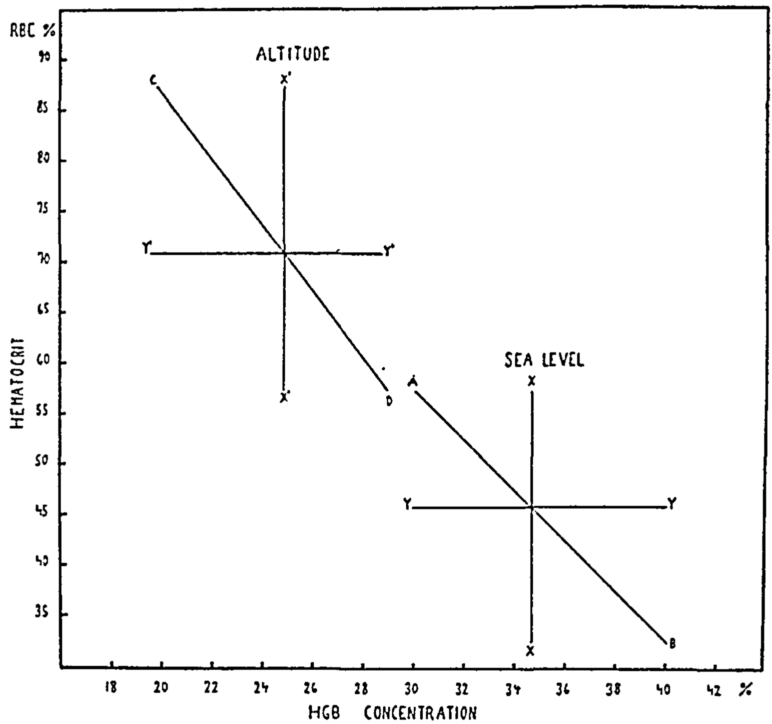

Fig. 3. Calculated regression lines for cell hemoglobin concentration on hematocrit (R.B.C. per cent), at high altitude and at sea level. *AB* is the calculated regression line of cell hemoglobin concentration on hematocrit at sea level. *XX* gives the mean of cell hemoglobin concentration, and *YY* the mean of hematocrit determinations, both at sea level. (Calculated on the basis of Osgood and Wintrobe and Miller's normal series.) *CD* is the calculated regression line of cell hemoglobin concentration on hematocrit at high altitude. *X'X'* is the mean of cell hemoglobin concentration, and *Y'Y'* the mean of the hematocrit determinations, both at high altitude.

Emmons (16), such an arrangement is quite advantageous for oxygen interchange. It is interesting to mention that Barcroft and his associates (11) in their investigations at Cerro de Pasco observed a definite increase in the oxygen binding property of the blood of these natives.

Viscosity determinations. We have determined the viscosity of the blood in 113 normal Indian natives. The Hess viscosimeter was employed in all

determinations, and distilled water used for comparison. The temperature of the room in which these determinations were made was always within 17 and 23°C.

The mean value of these determinations is 8.6 ±0.138 with variations between 5.2 and 15.2. The standard deviation is 2.2 ±0.096 and 82 per cent of the results fall between 6.0 and 9.9 (the normal average at sea level is given as 5.1 with variations between 4.7 and 5.9). The viscosity of the serum and plasma has been determined in seven and nine cases respectively. The average value obtained for serum viscosity is 1.95 and all the results fall strictly within normal limits. The total serum proteins were determined in 13 cases by means of the refractive index giving an average of 7.81 grams per cent. It is interesting to mention that the viscosity of the plasma normally exceeds that of the serum by 0.2 to 0.3 while at high altitude this relationship is inversed.

There was no relationship between the serum and plasma viscosity and the level of the red cell count. On the other hand there is an evident relationship between the erythrocyte count and the blood viscosity. The correlation is nonlinear as shown by the $\rho\, n^2 - r^2$ which is higher than three times its probable error. It appears that there is no correlation between the viscosity of the blood and the corpuscular hemoglobin. The increase in the viscosity of the blood found in all cases (but one) must depend chiefly on the increased cell volume, as we have demonstrated that the viscosity of the serum falls within normal limits.

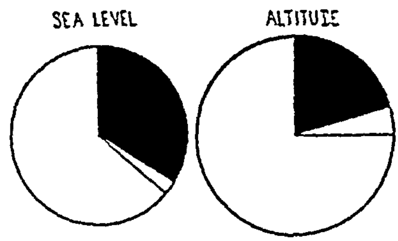

Fig. 4. Schematic representation of the normal erythrocyte at sea level and at high altitude. The red cell at high altitude has a larger size (given by the total circumference); it contains less hemoglobin (black and white areas within radius), and it is less saturated with oxygen (black area). The result is a larger surface area for the hemoglobin and oxygen content.

Fragility of the red cells. We have determined the fragility of the red cells in 14 normal Indian natives. The method employed was identical in all cases, using the same pipettes and solutions. Eight tubes of different concentration hypotonic salt solutions were freshly prepared. Blood was taken from one of the arm veins with the subject in the prone position and after a prolonged rest. In each tube a similar sized drop of blood was placed, and the observation carried out in the first few hours to avoid bacterial growth.

The average results of the fragility determinations are given in table 1. Five of the cases showed a minimal and maximal hemolysis at 0.38 and 0.28 per cent respectively; five cases at 0.28 and 0.20 per cent and finally three cases at 0.20 and 0.12 per cent. These observations indicate a definite

increased resistance of the red cells to hypotonic salt solutions, that is, a lesser tendency to hemolysis.

There was no relationship between the fragility of the erythrocytes and the level of the red cell count.

The constancy of these results seems to indicate that this increased resistance of the red cells is one of the definite characteristics in the blood picture of these natives. Although it will be quite impossible to account for all the factors which may have some relation to this increased resistance (a multiplicity of factors such as osmosis, surface tension, blood gases, temperature, cell volume, age of the cells, viscosity, etc., has been mentioned to affect the fragility of the erythrocytes) it seems most likely that it is related to an active bone marrow which sends into the circulation young cells. The same observation has been discussed by Minot and Buckman (17) in cases of Polycythemia Vera, and these authors call attention to the fact that young cells do not necessarily mean reticulated cells. That the blood of these Indian natives shows definite evidence of bone marrow activity, and the presence of young cells in the circulation has already been demonstrated by the Cerro de Pasco Expedition (11) and Hurtado and Guzmán Barrón (18).

It is interesting to mention that an increased amount of cholesterol in the blood has been related, by various observers, to an increased resistance of the red cells, but determinations of this substance in six normal natives gave values within normal limits.

Bilirubin determinations. We investigated the Van den Bergh reaction in 12 normal Indian natives, and the quantity of the plasma bilirubin was determined in another eight cases. The average results obtained are presented in table 1. All observations have been made on plasma of venous blood taken with the necessary precautions. In 12 determinations the indirect Van den Bergh reaction was positive in 11 cases (91 per cent), and eight of them gave just a faint reaction but enough to be clearly appreciated. In three cases the reaction was more intense and in one case resulted negatively. There was no relationship between this reaction and the red cell count, or the fragility of the red cells.

Accepting 0.5 mgm. per 100 cc. of plasma as the highest limit for the normal amount of bilirubin at sea level, we observed that six out of the eight cases in whom an actual determination of bilirubin was made, gave results slightly higher than the normal average. The highest increase obtained was 1.1 mgm. and the average of all determinations was 0.7 mgm.

The almost constant positive indirect Van den Bergh reaction and the finding of an increased amount of bilirubin in the blood plasma of these natives, suggest that the rate of cell destruction is somewhat marked. At high altitude there is, as a rule, an increased production of red cells by an active bone marrow, and opposed to this process, which if left undisturbed

would result in a quite pathological concentration of cells in the blood, there is an increased rate of destruction with the possible rôle of compensating such an increased activity. That the reticulo-endothelial system is overactive at high altitude is suggested by the presence of cells belonging to this system in the peripheral blood, as we will see later.

We have investigated the presence of urobilin in the urine by the spectroscopic method. In 20 specimens of urine, obtained from an equal number of normal men, we found in 19 of them a very faint trace of this pigment. The constancy of finding urobilin in the urine seems to exclude the idea that it may be due to liver dysfunction. In a whole year of the medical clinic at the hospital in Morococha we found but a very few cases of clinical liver insufficiency. In order to have further evidence on this matter we made a liver function test in three natives (all of whom had a positive indirect Van den Bergh reaction in the plasma and a faint trace of urobilin in the urine). Two milligrams of tetrabromosulphonphthalein per kilo body weight were injected intravenously, and after half an hour no trace of this dye was found in the plasma, indicating a normal removal by the liver cells. In all probabilities the slightly positive finding of urobilin in the urine of these natives is explained on the basis of the increased production of bilirubin. Urobilin has also been found in the urine in cases of Polycythemia Vera (17) without evidence of liver dysfunction.

Coagulation time of the blood. The coagulation time of the blood was determined in 95 normal Indian natives. A drop of a freely flowing blood from a deep skin puncture was placed on a clean slide observing the clot formation every half a minute. Although quite a simple procedure it gives consistent results when carefully done. Normal values with this method are given within 7 and 9 minutes.

The mean value of these 95 determinations is 6.5 ±0.120 minutes, with a standard deviation of 1.7 ±0.048 minutes, and a rather large coefficient of variation of 26.3 per cent. The variations range from 3.5 to 13 minutes, but 82 per cent of the results fall between 4 and 7.5 minutes. The mean value of 6.5 minutes is slightly lower than the normal average at sea level, but the difference is so small that we may only say that there is a tendency for a rather short coagulation time at high altitude. This tendency may be due to the increased viscosity of the blood, as it has been said that the greater the viscosity the less the coagulation time. The correlation coefficient between the coagulation time and the blood viscosity is low, but slightly above three times its probable error and with a negative sign which means some inverse correlation between these characteristics. There is no correlation between the coagulation and the level of the red cell count.

Leucocyte count and formula. The leucocyte count has been determined in 120 normal Indian natives. The determinations were made in the

morning, and standard pipettes used. The average of two counts was taken as the final result in every case.

The results varied between 2,300 and 9,300 white cells per cubic millimeter, with a mean value of 5,210 ±84.5. Eighty-seven per cent of the determinations fell between 3,000 and 6,900 cells. These results indicate that the white cell count in these natives varies within normal limits but with a tendency to be low. There was no correlation between the red cell count and the white cell count.

The leucocytic formula[4] was determined in 53 normal Indian natives and the average formula from these determinations is given in table 1. The average of the polynuclear cells fell within the normal limits of sea level. More detailed study showed that in 32 per cent of the cases the percentage of these cells was above the normal upper limit (75 per cent), and in 6 per cent below the normal limit (55 per cent). It appears to be a frequent occurrence to find a relative polynucleosis at high altitudes.

The average of the lymphocytic cells also fell within normal limits compared to sea level values. But in 17 per cent of the cases the percentage was above the normal upper limit (35 per cent), while in 19 per cent of the cases it came below the lower normal limit (15 per cent). There seems to be an equal tendency for a relative lymphocytosis, or a low percentage of lymphocytic cells.

A decrease in the eosinophilic cells appears to be evident from this study. The average value of 0.3 per cent is quite low and in 75 per cent of the cases no cells of this type were found in the routine count of the leucocytes.

The monocyte average percentage is equal to the sea level normal value. In 26 per cent of the cases there was a higher result than the upper normal limit (8 per cent), and in 32 per cent it came below the lowest normal limit of 3 per cent. In the cases with a high percentage of monocytes a considerable increase was sometimes found: in one case 18 per cent and in another case 24 per cent.

In 15 cases, or 28 per cent, very young or immature polynuclears were found. They are probably related to the overactive bone narrow. In no case nucleated red cells were observed.

The presence of histiocytes in the peripheral blood is an interesting fact. These cells were found in 21 cases, or 40 per cent, and their number varied between 1 and 7 cells per 100 leucocytes. There is some evidence to indicate that the histiocytes are cells derived from the reticulo-endothelial system. Their presence in the peripheral blood is considered to be a sign of alteration or overactivity of this system (Dameshek, 19). We may correlate this finding with the increased production of bilirubin and with the not uncommon high monocytic percentage in the leucocyte count.

[4] The writer is indebted to Dr. A. Guzmán Barrón for the determination of the leucocytic formulae.

SUMMARY AND CONCLUSIONS

A number of observations on certain of the blood characteristics of the Indian natives of the Peruvian Andes have been presented in this work. The investigation has been conducted in the town of Morococha, Peru, at an altitude of 14,890 feet, and with an average barometric pressure of 410 mm. Hg. The subjects studied have been carefully selected as normal men, and the results obtained suggest the following conclusions:

1. Observations on the erythrocyte count gave an average increase of about a million red cells per cubic millimeter over the sea level normal value, but a considerable percentage of these natives live with a red cell count which would be considered normal at sea level. This finding indicates that an increase in the erythrocytes of the circulating blood, although the common finding, is not an essential adaptation process for normal life at high altitudes. There is no relationship between the red cell count and age or body measurements.

2. The average number of grams of hemoglobin per 100 cc. of blood has almost the same value as the normal average at sea level. In only 50 per cent of the cases was a higher result obtained.

Each red cell at high altitude contains less hemoglobin, as compared with the normal erythrocyte at sea level, but the corpuscular hemoglobin is not a fixed characteristic: it varies inversely with the level of the red cell count, that is, the higher the count the less hemoglobin in each cell and vice versa.

Oxygen determinations in arterial and venous blood verify these findings, and indicate that the red cell comes to the tissues with a lower oxygen content as compared with the erythrocyte at sea level.

3. There is a marked increase in the hematocrit (RBC per cent), and it is proportionally greater than the increase in the red cell count, with the result that each erythrocyte has a larger volume (as compared with the normal corpuscle at sea level). Again the corpuscular volume is not a fixed characteristic: it varies inversely with the level of the red cell count.

The larger size of the erythrocytes has also been confirmed by direct measurement of its diameter.

4. The diminished amount of hemoglobin in a larger corpuscle gives a lower value for the concentration of that substance in relation to the volume of the cell. Only about one-fourth of the erythrocyte is saturated with hemoglobin. Equal and parallel changes in cell volume and hemoglobin, under the influence of variations in the red cell count, keep remarkably constant such concentration.

There is an almost perfect inverse correlation between the hematocrit (RBC per cent) and the hemoglobin concentration in each red cell.

5. The above findings suggest that the adaptation processes to the high

altitude, from the point of view of blood morphology, are to be found not primarily in red cell count and hemoglobin increases, as it has often been remarked, but rather in a fine and close correlation between cell number, volume and hemoglobin, and in the existence of a larger surface area in a given volume of blood and in the individual erythrocyte for the hemoglobin and oxygen content, factors which would favour the proper supply of this gas to the tissues, this last process being perhaps the basic and fundamental problem at high altitude.

6. The viscosity of the blood is definitely increased, whereas that of the plasma and serum remains strictly normal.

7. The red cells show a marked increase in the resistance to hemolysis. This is most likely related to the presence of young erythrocytes produced by the overactive bone marrow.

8. A positive indirect Van den Bergh reaction was obtained in most cases examined, and actual determinations of the plasma bilirubin gave results slightly above normal. This finding may be related to an increased rate of cell destruction, perhaps a compensatory process to balance the increased formation of red cells.

9. The blood coagulation time has a slight tendency to be shortened at high altitude. It has some inverse relation to the degree of the blood viscosity.

10. The average leucocyte count in these natives is essentially normal, but with a tendency to be low.

11. The average leucocytic formula falls within normal percentages of sea level values, with the exception of a decrease in the eosinophiles. Not infrequently a relative polynucleosis and monocytosis is found.

Young polynuclears cells are frequently observed. In a considerable number of cases histiocytes are found in the peripheral blood, indicating perhaps an overactivity of the reticulo-endothelial system.

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CHANGES IN MUSCLE GLYCOGEN ACCOMPANYING PHYSICAL TRAINING

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The purpose of this research was to study the effect of graded muscular exercise upon the glycogen content of the muscles of dogs. Several studies of a similar nature have been carried out previously. Rogozinski (1906) found that after 18 days' training the glycogen content of the muscles of 2 dogs was not above "normal." His controls were animals receiving the same food and care, but no training. Embden and Habs (1927), using rabbits, made very extensive investigations of the glycogen in the muscles of legs exercised by faradic stimulation of the corresponding sciatic nerve. Glycogen determinations made on the corresponding muscles of the opposite, unexercised leg provided control values. The results of these experiments show that the discrepancy between the glycogen content of the trained and untrained muscles increases progressively from the commencement of the training period. Hoffmann and Wertheimer (1927) reported that the glycogen content of denervated muscles is increased by electrical stimulation of the muscle, even though atrophy occurs. Palladin and Ferdmann (1928) confirmed the findings of Embden and Habs, using the same species of animal and the same method of training.

The experiments of Embden and Habs, which we have had no difficulty in repeating, are very complete and the results are clear cut. These results establish the fact that the glycogen content of skeletal muscles can be increased by the procedure they used. It was thought, however, that it would be of interest to investigate the same phenomenon in animals trained under more physiological conditions; that is, when the animals exercised themselves by natural muscular movements.

Methods. Strong, healthy dogs were trained to run on three legs by tying one of the hind legs loosely, by means of a tape, to a strap about the animal's abdomen. The animals very quickly learned to run easily on three legs, the fourth being held in the characteristic position seen in a lame dog. Little or no tension was exerted on the tape holding the fourth leg. The exercising took place in a wooden wheel about 5 feet in diameter. This wheel could be turned by a motor, but the animals generally propelled it by their own movements. The exercise periods, of about 30 minutes'

length, were repeated 4 to 6 times each day for a total training period of from 7 to 42 days. When not in the wheel the animals were free to run on all four legs in roomy cages.

Twenty-four hours after the end of the training period the animals were gently anesthetized by intravenous injection of sodium amytal. Several muscles of both hind legs were carefully removed. Duplicate samples of 2 to 5 grams were taken from each muscle for glycogen determination by Pflüger's method and about 1 gram was used for estimation of dry weight. Bilaterally symmetrical muscles were removed practically simultaneously and the samples were immediately dropped into hot KOH.

The glycogen content of the muscles of the unexercised leg furnish control values since, as first shown by Cramer (1888), the glycogen content of the bilaterally symmetrical muscles is very nearly identical under normal conditions. Illustrations of this close relationship are available in the work of Best, Hoet and Marks (1926) and of Embden and Habs (1927). As a result of a large number of determinations which we have made it has been found that the glycogen contents of bilaterally symmetrical muscles usually do not show differences of more than 10 per cent, and never more than 20 per cent in normal, healthy dogs. A difference of more than 20 per cent can therefore be considered as significant. The error of the glycogen method is not greater than 10 per cent.

We have checked the possible effect of long indoor confinement upon the animals by training dogs which had been in the laboratory several months, as well as those which had been in for only a short time. All animals were fed on dog biscuits. While it is very difficult to measure the maximum ability of a laboratory animal to perform work, it was perfectly obvious that all the dogs were able to run more efficiently as a result of the training. It is our opinion that the animals trained for the longer periods were in excellent condition, well trained and willing to work.

Experimental results. Many of the muscles of the hind leg do not seem to be affected by the type of exercise employed in the investigation. Several of the muscles of the thigh have a large fat content which interferes with accurate determinations of glycogen. Others cannot be removed easily and rapidly. Considering these factors, but more particularly the character of the exercise, we chose the tibialis anticus, the gastrocnemius, the rectus femoris and the vastus lateralis as the most suitable muscles for our study. After examining a cinematographic record of an exercising animal, Professors Watt and Cates of the Department of Anatomy, who were not familiar with the results we had already obtained, concluded that the muscles mentioned above should be most affected by the work performed. Of these muscles the one giving the most consistent results is the rectus femoris. The vastus lateralis in most instances showed practically the same changes as the rectus femoris. The results of the

analyses of the rectus femoris and the vastus lateralis are included in table 1. The change in the tibialis anticus was almost always in the same direction, but to a lesser extent than that of the rectus femoris. The gastrocnemius sometimes showed a large increase in the glycogen content

TABLE 1

Effects of training period of from 7 to 42 days on muscle glycogen

Glycogen content as percentage of wet weight

EXPERIMENT	DAYS OF TRAINING	UNTRAINED MUSCLE	TRAINED MUSCLE	DIFFERENCES	
				Found	Percentage
			Recti femoris		
A 7	7	0.76	1.09	0.33	43.5
D 1	7	1.16	2.16	1.00	86.0
A 3	10	0.61	0.88	0.27	44.5
A 5	10	0.90	1.19	0.29	32.0
A 2	10	1.00	2.15	1.15	115.0
D 7	12	1.08	1.37	0.29	27.0
D 2	14	0.88	1.21	0.33	37.5
A 6	16	1.18	2.14	0.96	81.0
D 8	21	0.57	0.70	0.13	23.0
D 4	23	1.54	1.79	0.25	16.0
D 5	24	0.96	1.52	0.54	58.5
A 10	31	1.09	1.09	0.00	0.0
A 1	35	0.72	0.78	0.06	8.5
D 3	42	1.16	1.13	0.03	−2.6
A 15	42	0.33	0.32	0.01	−3.0
			Vasti lateralis		
D 1	7	1.13	1.98	0.85	75.0
D 7	12	1.02	1.33	0.31	31.0
D 2	14	0.72	1.05	0.33	46.0
D 8	21	0.66	0.65	0.01	−1.0
D 4	23	1.42	1.51	0.09	6.3
D 5	24	0.84	1.38	0.54	64.0
D 3	42	1.10	1.28	0.18	16.3
A 15	42	0.44	0.32	0.12	−26.5

of the trained muscle, but the results were on the whole more irregular than in the other cases.

No values for dry weights are included, as in confirmation of other workers we have not found that a period of training produces any significant change in the water content of muscles.

In the accompanying table the glycogen contents of exercised and unexercised muscles taken from animals which had been trained for periods of

7 to 42 days, are given. The results confirm, in certain respects, the work on rabbits. Training periods up to 15 or 16 days cause an appreciable and often striking increase in the glycogen content of the trained muscles. They suggest, in addition, that with continued training the differences between the trained and untrained muscles tend to disappear, so that when the training period has been extended to 5 or 6 weeks, the glycogen contents of the muscles are in some cases as similar as they were before training commenced. The figures also suggest that the diminution and final disappearance of the difference in glycogen content between trained and untrained muscles is brought about by a return of the glycogen of the trained muscle to the pre-training level. The results indicate that there may be a rather broad optimum period of training for the accumulation of glycogen in the muscles. It is clear that in the case of the rectus femoris muscle training periods up to 16 days show in every case increases in the glycogen content of the trained muscle. These increases are from 1¼ to 5 times as large as differences ever found in healthy, normal animals. As the training period is extended beyond 16 to 20 days, the differences between trained and untrained recti, with one notable exception, become less than that exhibited by the muscles of other animals at the end of a shorter period of training. In the four animals whose training periods varied from 31 to 42 days, the increases have entirely disappeared, and both trained and untrained muscles show an equal and relatively low glycogen content. More consistent results might have been secured if animals of uniform type, age and aptitude for the work had been available.

In certain cases an attempt has been made to determine the buffering power of the trained and untrained muscles. The technique elaborated by Irving and his collaborators in this laboratory has been used. The results suggest that the trained muscles may have a greater buffering capacity, but further work is necessary to determine the significance of the changes. No significant difference in the phospho-creatine contents of the trained and untrained muscles has been detected under the conditions of our experiments. Ferdmann and Fernschmidt (1929) have reported that the phospho-creatine of the recti femoris muscles of rabbits may be increased by the training procedure used by Embden and Habs (1927).

SUMMARY

Dogs exercised for from two to three weeks show a considerable increase in the glycogen content of certain of the trained muscles. The results, in the case of the rectus femoris muscle, suggest that there may be an optimum period of training for the accumulation of glycogen. Since muscles trained for longer periods, in which there is no increase in glycogen, appear to retain their increased ability to perform work, a raised glycogen

content of skeletal muscle does not appear to be an essential factor in the increased efficiency which results from training.

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FURTHER OBSERVATIONS OF RAPID GROWTH OF THE ALBINO RAT[1]

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Aside from the interest in fundamental considerations of the process of growth, observations of increasing rates of development have a practical bearing upon biochemical studies involving the use of laboratory animals. The albino rat is currently employed in investigations of widely varied character including pharmacological and vitamin assays, determination of the biological value of foodstuffs and metabolism. In many studies of this nature the main, if not the only, criterion of response to the experimental conditions imposed, is the rate of growth. If the optimal development of this species is a changing feature of its biometry, it is obvious that each experiment should contain its own so-called control. The "normal" curve of growth is not fixed but, as will be shown later, apparently changes with nutritive conditions and, possibly, with genetic factors.

The records of the optimal rates of growth of the laboratory rat indicates a definite upward trend. In 1906 Donaldson, Dunn and Watson published data showing that the average weight of the male albino rat at 100 days of age was 165 grams. Nine years later King (1915) demonstrated that at this age animals of the same sex weighed 200 grams. Within the next thirteen years Smith and Bing (1928), using rats originally derived from the Wistar Institute strain, observed an average weight of 315 grams in males 100 days old. Similar records of increased rates of growth in various other stock colonies at the present time indicate that this change in development is a general phenomenon for the albino rat.

Unusually rapid development was demonstrated in a smaller number of rats by Osborne and Mendel (1926). The ration, consisting of purified food materials, was adjusted so that the proportion of protein was increased and liberal allowances of adjuvants such as liver, dried yeast and lettuce were provided. Whereas the previous record time for increase in body weight from 60 to 200 grams had been 63 days, fourteen of these rats

[1] The expenses of this investigation were shared by the Connecticut Agricultural Experiment Station and the Carnegie Institution of Washington, D. C. A preliminary account of this study was presented to the National Academy of Sciences in New Haven, November 17, 1931.

showed this change in weight in an average time of 26 days. Later it was stated (Mendel and Cannon, 1927) that the average time required for 20 of the best of these rats to grow from 60 to 300 grams was 46 days, an average daily gain of 5.3 grams. The present report records the most rapid growth of the albino rat yet observed as far as we are aware. It is of particular significance because the animals were of the same strain and were housed in the same room in the biochemical laboratory of the Connecticut Agricultural Experiment Station as were those of Osborne and Mendel (1926). Heredity and environmental conditions, except for the ration, were therefore the same.

The animals used for this study were males obtained from parents which formed part of an extensive breeding investigation. A large proportion of the young weighed from 40 to 55 grams at weaning (21 days of age). For the rapid growth experiment, individuals were selected from the lower portions of the range in body weight as well as from those with the higher weights. The rats were kept in separate cages and the care conformed in general to that discussed by Smith, Cowgill and Croll (1925).

Food and water were given ad libitum. The following dry ration[2] (97 per cent) was mixed with 3 per cent cod liver oil: linseed oil meal 15 per cent, corn meal 20 per cent, ground malted barley 10 per cent, red dog flour 22 per cent, dried skim milk 12 per cent, oat flour 15 per cent, soluble blood meal 3 per cent, sodium chloride 1 per cent, ground limestone 1 per cent, steamed bone meal 1 per cent. The moisture content of the air-dry mixture was 57 per cent, the protein[3] 22.1 per cent and the calcium 0.92 per cent. In addition a paste food containing whole milk powder 25 per cent, casein 25 per cent, wheat embryo 20 per cent and lard 30 per cent was freely available at all times. The moisture content of this air-dry food was 2.2 per cent, the protein[3] 34.9 per cent and the calcium 0.27 per cent. Each rat received 20 grams lettuce per diem and 3 grams dried yeast twice a week. Because of the mealy character of the dry ration and the consequent spilling, the food intake records are not considered accurate. In view of the analyses of the foods, it appears that the animals consumed somewhat more protein than is contained in the ordinary adequate experimental rations. In general, the best records of growth observed by Osborne and Mendel (1926) were made by the animals on the higher protein intakes. Besides the liberal allowance of calories and protein, our experimental diet provided an abundance of vitamin supplements and mineral salts.

[2] Calf meal approved by the College Feed Conference Board and marketed by the Coöperative G. L. F. Exchange, Inc., Buffalo, N. Y. See Maynard, L. M., Science *71*, 192, 1930.

[3] These values are calculated from the total nitrogen of the air-dry material using the factor 6.25.

The data for the rates of growth of the rats in the present experiment are presented in table 1. The number of days required to make a given increase in body weight as well as the average daily gain in weight are given. It should be emphasized that in each of the selected periods of growth, the

TABLE 1

Rate of growth

RAT NUMBER	WEANING WEIGHT	60 TO 200 GRAMS		60 TO 300 GRAMS		60 TO 400 GRAMS		60 TO 500 GRAMS		MAXIMUM BODY WEIGHT	AGE AT MAXIMUM BODY WEIGHT
		Time required	Average daily gain	Time required	Average daily gain	Time required	Average daily gain	Time required	Average daily gain		
	gms.	*days*	*gms.*	*days*	*gms.*	*days*	*gms.*	*days*	*gms.*	*gms.*	*days*
C3120	60	20	7.0	33	7.3	53	6.4	83	5.3	535	161
C3397	44	20	7.0	33	7.3	50	6.8	71	6.2	737	231
C3417	44	19	7.4	33	7.3	51	6.7	78	5.7	580	140
C3290	52	22	6.4	35	6.9					393	70
C3398	50	24	5.8	36	6.7	53	6.4	79	5.6	600	161
C3119	52	24	5.8	37	6.5	71	4.8	140	3.1	540	203
C3342	42	21	6.7	37	6.5	86	4.0			450	175
C3241	40	21	6.7	38	6.3	59	5.8	106	4.2	509	168
C3407	40	22	6.4	38	6.3	67	5.1	106	4.2	620	238
C3263	50	23	6.1	39	6.1	60	5.7			470	147
C3327	54	22	6.4	40	6.0	63	5.4	103	4.3	535	168
C3406	53	27	5.2	41	5.9	68	5.0	131	3.4	507	161
C3326	41	23	6.1	42	5.7	68	5.0	116	3.8	600	231
C3328	52	25	5.6	43	5.6	76	4.5			474	168
C3393	44	27	5.2	44	5.5	107	3.2			450	161
C3264	54	23	6.1	45	5.3	64	5.3	131	3.4	595	266
C3242	42	24	5.8	47	5.1	77	4.4			454	154
C3118	53	24	5.8	47	5.1	92	3.7			407	147
C3392	40	30	4.7	48	5.0	90	3.8			482	168
C3421	55	24	5.8	68	3.5					375	154
C3544	60	25	5.6	41	5.9	58	5.9	85	5.2	658	158
Mean.......		23.3	6.1	41.1	6.0	69.1	5.2	102.4	4.5		
Standard deviation..		±2.55	±0.65	±7.52	±0.90	±15.19	±1.02	±22.48	±0.99		
Probable error......		±0.38	±0.10	±1.10	±0.13	±2.35	±0.16	±4.51	±0.198		
Coefficient of variation.......		11.16	10.65	18.29	15.00	22.00	19.62	21.95	22.00		

record of *all* of the rats then living have been used in the statistical evaluation. In tables 2 and 3 comparisons are made between the growth rates of these animals and the twenty rats studied by Mendel and Cannon (1927) which had been selected from a larger group because of their rapid

TABLE 2

Time required for increase in body weight of albino rats

NUMBER OF RATS	MEAN TIME	σ	COEFFICIENT OF VARIATION	PROBABLE ERROR	SOURCE OF DATA	
From 60 to 200 grams body weight						
	days					
21	23.3	±2.55	11.16	±0.38	Anderson-Smith.	D = 2.6
20	25.9	±1.98	7.65	±0.30	Calculated from data furnished by Prof. L. B. Mendel	PE_D = ±0.48 SR = 5.4
From 60 to 300 grams body weight						
21	41.1	±7.52	18.29	±1.10	Anderson-Smith.	D = 4.7
20	45.8	±3.33	7.27	±0.50	Calculated from table 1, Mendel and Cannon, 1927	PE_D = ±1.21 SR = 3.9
From 60 to 400 grams body weight						
19	69.1	±15.19	22.00	±2.35	Anderson-Smith.	D = 26.0
12	95.1	±16.75	17.61	±3.23	Calculated from data furnished by Prof. L. B. Mendel	PE_D = ±4.08 SR = 6.4
From 60 to 500 grams body weight						
12	102.4	±22.48	21.95	±4.51	Anderson-Smith	

σ (Standard deviation) $= \sqrt{\frac{\Sigma d^2}{n}}$.

Coefficient of Variation $= \frac{\sigma \times 100}{\text{mean}}$.

PE (Probable Error) $= \frac{\sigma \times 0.6745}{\sqrt{n}}$.

PE_D (Probable Error of Difference) $= \sqrt{(\text{PE mean } 1)^2 + (\text{PE mean } 2)^2}$.

SR (Significance Ratio) $= \frac{D}{PE_D}$.

Σd^2 = sum of deviations from mean, squared.
n = number of observations.
D = difference between means.

development and which, up to the present, had shown the most rapid rate of growth recorded for this species.[4]

[4] We are indebted to Professor L. B. Mendel for unpublished data bearing on the growth of these rats.

The time required to make the increases in body weight of 140, 240 and 340 grams was, in every case, less for the rats of the present study than for those of Mendel and Cannon. Likewise the daily gain was greater for the former group. In all comparisons the significance ratio was of such magnitude that there is no doubt that, measured by the criteria employed, the rats of the present study are definitely superior to the best group on record up to this time. As an example of extraordinary gain in weight under

TABLE 3

Daily gain in body weight of albino rats

NUMBER OF RATS	MEAN GAIN PER DIEM	σ	COEFFICIENT OF VARIATION	PROBABLE ERROR	SOURCE OF DATA	
From 60 to 200 grams body weight						
	gms.					
21	6.1	±0.65	10.65	±0.10	Anderson-Smith.	$D = 0.6$
20	5.5	±0.41	7.52	±0.06	Calculated from data furnished by Prof. L. B. Mendel	$PE_D = \pm 0.12$ $SR = 5.0$
From 60 to 300 grams body weight						
21	6.0	±0.90	15.00	±0.13	Anderson-Smith.	$D = 0.7$
20	5.3	±0.40	7.55	±0.06	Calculated from table 1, Mendel and Cannon, 1927	$PE_D = 0.14$ $SR = 5.0$
From 60 to 400 grams body weight						
19	5.2	±1.02	19.62	±0.16	Anderson-Smith.	$D = 1.5$
12	3.7	±0.75	20.27	±0.15	Calculated from data furnished by Prof. L. B. Mendel	$PE_D = 0.22$ $SR = 6.8$
From 60 to 500 grams body weight						
12	4.5	±0.99	22.00	±0.198	Anderson-Smith.	

laboratory conditions without recourse to growth stimulants other than those in the diet, the performance of rat C3120 can be cited. During the four weeks following weaning (21 days of age) this individual grew at the rate of 8 grams a day; for the last half of this period the rate was 9.5 grams a day. This rat weighed 282 grams at 49 days of age, whereas the maximum weight attained by males in the rapidly-growing colony studied by Smith and Bing (1928) was 205 grams at this age.

In tables 2 and 3 it is evident that the variability of the data is less for the rats of Mendel and Cannon than in those of the present experiment. This is probably accounted for by the fact that the former group represents individuals *purposely selected* for their rapid growth. The superior performance of the present series of animals is thereby accentuated inasmuch as it is based upon purely random sampling of data.

Attention is called to the maintenance of rate of growth at a high level throughout the first and second period in both groups of animals. Furthermore, in the rats of the present study, the average gain per diem from 60 to 400 grams was maintained in the remarkable value of 5.2 grams and in this interval only two individuals had ceased to contribute to the average. In view of the fact that increase in body weight ordinarily diminishes in these upper ranges, the conclusion might be drawn that when the nutritional environment is made more favorable, there is a tendency for the self-inhibitory stage of development to be postponed.

When after growth has been suppressed through a dietary deficiency, the ration is made satisfactory, growth is frequently resumed at an exceedingly rapid rate. The greatest average daily rate of such "growth of recovery" recorded by Mendel and Cannon (1927) was 7.6 grams over an interval of 14 days, during which time the rat increased in body weight from 86 to 192 grams. In our group of animals the maximum average daily rate of growth—from 148 grams to 282 grams body weight—was 9.5 grams over a period of 14 days. It thus appears at present that under optimal nutritive and other environmental conditions, the extremely rapid gains frequently observed during realimentation are not necessarily unattainable under conditions of uninterrupted growth.

Mendel and Cannon (1927) showed that the records for growth made by their animals compared favorably with those published by Evans (1923–24) and obtained with rats which had received injections of hypophyseal extracts. In a recent study Bryan and Gaiser (1932) have facilitated growth both by administration of pituitary extract and by feeding rations known to favor rapid development. Young male rats were injected with an extract of the anterior pituitary; daily doses varying progressively from 0.5 to 2.00 cc. were given intraperitoneally. One group of rats was maintained on one of the rations employed by Osborne and Mendel (1926) in their study of rapid growth while a second group was given a ration composed of whole milk powder, ground wheat and minerals. The growth on the former diet was superior but in both cases the injected animals grew more rapidly than the non-injected controls. The average daily rate of growth from 60 to 200 grams shown by *fifteen of the best growers* selected from the animals on the better ration was 6.1 grams with the maximum at 7.4 grams. In the present study the average daily gain for 21 *unselected rats without growth stimulants other than those in the ration*

was likewise 6.1 grams with a maximum of 7.4 grams. Bryan and Gaiser (1932) state that "the data at hand seem to indicate that the maximum daily increase in weight of which a young male rat is capable over a considerable period of days lies in the neighborhood of six or seven grams." We call attention to rat C3120 in our series which for 28 days following weaning maintained an average daily gain of 8.0 grams and for the last 14 days of that time increased 9.5 grams per diem in body weight. It thus appears that the limit of growth has not yet been reached, especially if the growth-promoting substance of the pituitary should be used in conjunction with more favorable rations and improved strains of rats.

The maximum body weights and the age at which they were attained are given in table 1. At the present time four of the rats are still growing. Rat C3397, at 231 days of age weighs 737 grams; no. C3407 at 238 days of age, 620 grams; no. C3264 at 266 days of age weighs 595 grams and rat C3544 at 158 days of age weighs 658 grams. Whereas it appears that the circumstances favoring a rapid rate of growth also induce a high maximum body weight, the data summarized in table 1 lead to the conclusion that, under the experimental conditions herein described, there is little, if any, correlation between weight at weaning and either rate of growth or maximum body weight attained. For instance, rat C3397, one of the largest specimens produced in this colony, which grew from 60 to 500 grams body weight in only 71 days and at 231 days weighed 737 grams, weighed 44 grams at weaning, a value at the lower range of body weights selected for this experiment. Conversely, some of the animals whose weights at weaning were in the upper range, did not excel in either rate of growth or final weight attained. These observations are not in harmony with those made by Hanson and Heys (1927). These investigators showed that the body weight of rats at birth and up to 20 days has an influence upon the weight at 100 days. However, it is recognized that the number of animals employed in the present study is too small to draw general conclusions applying to the species. Furthermore, the minimum weight at weaning of the present group of rats would be considered superior for many colonies.

Seasonal variation in growth has been observed by Hanson and Heys (1927). The male rats grew somewhat better in the fall months than at other times of the year. The diet was the Wistar Institute cooked food supplemented with lettuce and dog biscuit. On the other hand, Wu and Chen (1929) could demonstrate a difference only on their so-called vegetarian diet, growth being better in summer than in winter; on a ration containing a liberal proportion of whole milk powder, no seasonal variation was observed. In the present study all but two of the rats were born in May and June. The most rapid growth of the group as a whole took place, therefore, during the months of June, July and August, a season which, according to Hanson and Heys (1927), is least favorable. It ap-

pears from the limited data available from the present study that the influence of other environmental factors tends to be largely minimized when optimal nutritive conditions are provided.

The question might be raised as to whether the animals which have exhibited this rapid growth are normal in structure. Outhouse and Mendel (1931) compared rats which had shown very rapid development with others growing more slowly. The data indicate that when body weights somewhat above 400 grams are attained, individuals of the same size have identical body measurements and proportions, irrespective of rate development. In view of the fact that their animals were of the same strain and grown in the same laboratory as those of the present study, the conclusion seems warranted that the rats herein described were not unusual in body structure.

SUMMARY

The most rapid growth of albino rats yet recorded, as far as we are aware, has been obtained. These studies emphasize anew the possibility of facilitating the inherent capacity to grow by means of a favorable nutritive environment alone.

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A FUNCTIONAL ANALYSIS OF THE CERVICAL SYMPATHETIC NERVE SUPPLY TO THE EYE

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The sympathetic nerve supply to the eye and adjacent parts is of interest both as one component of the complexly coördinated visual mechanism and from the viewpoint of the manner of functioning of the sympathtic nervous system itself. The cervical sympathetic is one of the few structures where autonomic nerve fibers may be isolated from other types, and where at the same time both pre- and postganglionic regions are available for functional analysis by means of nerve action potentials. We report here the results of experiments in which pre- and postganglionic nerves in the cat and rabbit were stimulated at various rates, and at different intensities. Observations of the correlation between strength and frequency of stimulus and functional response of various structures in the body were made, and action potentials were recorded by means of the cathode ray oscillograph, either at the same time or immediately after excision of the nerves. At the end of the experiments the nerves stimulated were osmicated and sectioned. Further, carcasses of cat, rabbit, and monkey have been injected with 2 per cent formalin via the carotid after washing out blood with Ringer's solution, fixed in 1 per cent formalin and dissections made along the course of the sympathetic to the eye. This material was washed thoroughly, osmicated, and sectioned, including serial sections of the sympathetic ganglion and of the ciliary ganglion and its adjacent nerves. This histological work will be reported separately, but its results have been drawn on here as occasion demanded. It is hoped that we will be able to deal with the parasympathetic supply to the eye via the third nerve in a corresponding manner.

Motor responses to sympathetic stimulation were followed: nictitating membrane, Müller's muscle and pupil of the eye, vasoconstriction of the vessels of the conjunctiva and ear, and the pilomotor muscles between the ear and eye. No evidence of inhibition by the sympathetic has been obtained. Animals were anesthetized, or, preferably, decerebrated, since urethane at least appeared to render the actions studied sluggish. Decerebration was done under ether through a trephine hole, no vessels being tied off, in some cases the spoon being passed deeply enough to destroy the

parasympathetic reflex pathway to the pupil. When this was not done the pupil was so constricted by the light required for accurate observation, that we were not certain of detecting a trustworthy threshold for constriction. Occasional removal of the plug in the trephine hole relieved respiratory symptoms, during experiments lasting up to an hour.

In all cases our results depended upon accurate determinations of threshold. Condenser charges were used as stimuli, because easier to control than induction shocks, where variable interrupter contact is a disturbing factor. With the latter, a circuit breaker in the primary circuit must interrupt a large current, and even when the mechanism is behaving with apparent uniformity, variation in spark discharge introduces variations in the rate of interruption of the current, and therefore in form of the secondary shock. With condensers, the circuit breaker is in the nerve circuit, and the high resistance of this prevents any arcing at all with the small currents required, besides rendering slight variations in resistance of the contact insignificant. Thresholds were recorded in voltages, applied as charges through specified condensers, the discharges also passing through the tissue in the reverse direction, but with a leak resistance in the discharge circuit so high that no stimulation occurred. For end-organ threshold, where a single threshold nerve stimulus did not cause a response large enough to be observed, stimuli were repeated at 5-to-10-per-second rates for several seconds. The correspondence between the tissue response threshold so obtained and the nerve threshold for a given potential wave in the oscillographic record was very close, indicating that the two methods of detecting threshold were of about the same degree of sensitivity.

Anatomical. The technic of correlating functional responses to nerve stimulation in the body with the anatomical character of the fiber stimulated, depends upon work previously reported (Bishop and Heinbecker, 1930), indicating that the properties of nerve axons as measured by their action potentials correspond to their histological character as revealed in osmicated sections of the nerve. This differential correspondence has been demonstrated with respect to threshold, rate of conduction, chronaxie, and refractory period in nerves where the types of fibers were recognizable by reason of the presence or absence of one type. In the rabbit (and Belgian hare), the fibers of the preganglionic cervical sympathetic are of two types, small myelinated and unmyelinated,[1] all motor, and all synapsing

[1] The axons which we refer to as "unmyelinated" are those found in sympathetic, parasympathetic and mixed peripheral nerves, seen when stained with osmic acid as light greenish-gray rings, in contrast to the well-blackened rings of the small myelinated autonomic fibers of the same or slightly larger diameter. They range from 1 to 3 mu in size, and are otherwise quite similar in appearance to the small myelinated fibers, but differ unmistakably in threshold and conduction rate. Their sheaths can be counterstained after osmic with alizarin red and aniline oil, when they stand out

in the superior ganglion. The myelinated predominate in number, in the ratio of perhaps two to one. Postganglionically the ratio is quite different, the unmyelinated fibers predominating, at least ten to one. There are, in fact, so few postganglionic *myelinated* fibers synapsing with preganglionic myelinated that action potentials characterizing them are not certainly recognizable when stimulating pre- and leading postganglionically. There are, however, in the postganglionic stretch only, some larger fibers that must pass to the vagus. There appears no action potential characterizing them in response to preganglionic stimulating of the sympathetic, and anastomoses between the vagus and sympathetic nerves can be traced in sections at the ganglionic regions. On the other hand, both myelinated and unmyelinated preganglionic axons synapse with postganglionic *un*myelinated, as indicated by action potentials led off postganglionically as well as by histological evidence. One potential elevation, due to weak stimulation, and a second, due to stronger stimulation, which in the preganglionic stretch elicit separate potentials traveling at different rates, emerge postganglionically retarded by an interval due to preganglionic conduction and delay in the ganglion, but traveling at approximately the same rate behind the ganglion. Further, stimulation postganglionically gives no preganglionic response and causes but a single postganglionic elevation with a rate and threshold that are characteristic of unmyelinated axons. Measurements of rate of conduction and threshold thus give the same differential evidence for myelinated and unmyelinated axons here as in nerves previously studied (Bishop and Heinbecker, l.c.). All the response mechanisms studied in the rabbit are apparently innervated by postganglionic *un*myelinated axons.

While stimulation of the preganglionic stretch causes postganglionic response with a synaptic delay, and no conduction occurs in the reverse direction, it is certain that not all the unmyelinated axons in the preganglionic stretch are preganglionic fibers. In the cat, fibers synapsing in the superior ganglion course back along the sympathetic trunk and pass to the heart (Reighard and Jennings, 1901) and this presumably occurs in the rabbit. The superior cardiac nerves in the cat, monkey, and man and sympathetic cardiac branches of the chain in frog and turtle have been examined by us and found to consist almost exclusively of unmeylinated axons; in the cat, monkey, and man they give only one recognizable action potential elevation, conducting at less than a meter per second, characteristic of unmyelinated axons (work to be published elsewhere, Heinbecker and Bishop).

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more clearly. Their action potentials can be recognized unmistakably in nerves where they make up the bulk of the nerve's content and are practically the only fibers seen after osmication. They are then recognized in other nerves by action potentials having similar characteristics (Bishop and Heinbecker, 1930).

In the cat, similar procedures indicate a certain difference. The postganglionic stretch available between ganglion and bony canal is too short to give reliable conduction-rate data, but responses of different end organs to different thresholds of stimulation give criteria for recognizing different fiber types corresponding to the histological picture. Here, as in the rabbit, the preganglionic nerve has uniformly two types of axons, myelinated and unmyelinated, with properties as indicated by action potentials corresponding. Stimulations of both types preganglionically give corresponding potentials postganglionically, after a synaptic delay, and with no conduction antidromically. Recorded in the body, the first preganglionic nerve response with low threshold (meylinated axons) can be correlated with different end-organ responses (details below). Stimulation postganglionically now gives these same responses at much different thresholds, one characteristic of myelinated axons, the other of unmyelinated. This indicates that myelinated preganglionic axons in the cat synapse both with myelinated and with unmyelinated axons.

These results corroborate the anatomical findings. Postganglionically in the cat about one-third of the axons are myelinated axons of the sympathetic type. Other, larger, myelinated axons apparently go to the vagus sensory ganglion, as reported by Stöhr, and as we also find in sections of the ganglion region of the cat, as well as of the rabbit. Contrary to the condition in the rabbit, sensory vagus fibers in the sympathetic trunk of the cat in some cases pass caudally as well as forward. When present, they can be recognized by their action potentials, with lower threshold and faster conduction rate than typically sympathetic fibers. Here preganglionic sympathetic stimulation of these fibers alone gives no response postganglionically, and no response of the end organs studied.

The vagus fibers in the postganglionic sympathetic nerve presumably serve such structures as the carotid sinus. We find no separate branches running forward from the vagus nerve parallel to the sympathetic. Concerning those in the preganglionic sympathetic stretch we have more specific data. In the first place, this fiber group is not constant in the sympathetic of the cat, but varies from a number of fibers just sufficient to give a recognizable potential elevation, or none at all, to a number that gives an elevation as great in amplitude as that of any other one group in the nerve. The number of larger myelinated axons present corresponds roughly to the potentials (Heinbecker, 1930; Bishop and Heinbecker, 1930). Secondly when this group is absent, the preganglionic cross section in the cat has essentially the same fiber and potential picture as in the rabbit. Under these conditions, a separate fine nerve is present in the cat, resembling the depressor of the rabbit both in its anatomical structure and in its potentials, and traceable at its anterior end into the vagus. The vagus depressorlike fibers, therefore, are distributed variably between the sympa-

thetic, a separate depressor, and possibly the vagus itself in the cat, and similar fibers in both cat and rabbit come from head structures.

Physiological experiments have not been performed on corresponding structures in the monkey, except that preganglionic action potentials correspond in Macacus rhesus to those of the cat. Postganglionically many myelinated axons appear, mostly of the small sympathetic type, but some of the larger type suggest a vagus source. Many branches leave the ganglion to run laterally or posteriorly, and some of these consist exclusively of unmyelinated axons. The main trunk running forward along the internal carotid is being further investigated.

Considering these results together with those previously reported (Heinbecker, 1929) for the turtle, we find no support for Gaskell's dictum that preganglionic sympathetic fibers are always myelinated and postganglionic *un*myelinated. All three animals here studied have some preganglionic *un*myelinated fibers synapsing with similar fibers. Most if not all of the turtle's preganglionic *myelinated* fibers synapse with postganglionic *myelinated*, and the same is true of *some* of the myelinated axons in the cat. The rest of the fibers in the cat nerve, and practically all the preganglionic myelinated fibers of the rabbit nerve, synapse with *un*myelinated fibers. These three animals thus furnish every possible combination except that of preganglionic unmyelinated to postganglionic myelinated. These data apply only to the superior cervical ganglion. The presumption from the histological picture is that the monkey has relationships similar to those of the cat.

FUNCTIONAL. The conducted action potential record of the cervical sympathetic nerve shows the responses of different fiber groups separated out as more or less distinct waves by reason of differences of conduction rate, the slower fibers having in general the higher threshold. Some of these waves overlap at distances of conduction available in this nerve. The groups are better defined by their differences in threshold, which do not overlap significantly, so that, watching the various elevations increase as the stimulus is gradually increased, there is a relative pause between groups even if their records overlap temporally. Four or five centimeters of nerve were dissected out, including the ganglion and the postganglionic stretch up to its entrance into the cranium, all branches being cut. The relatively unbranched postganglionic length available for study in the cat is usually less than 1 cm., in the rabbit it is slightly longer, and in the Belgian hare, longer still. Metal electrodes were placed 4 mm. apart on the nerve pre- or postganglionically, the cathode toward the head (fig. 1). When records were taken on the oscillograph the current was reversed to bring the cathode toward the recording leads, since with strong stimuli the anode could either stimulate or block the response from the cathode. To exclude body disturbances to the oscillograph, which was operating at

a sensitivity of 200 meters per volt, a grounded metal electrode was inserted between stimulating electrode pair and leading-off metal electrodes, and the animal was then insulated otherwise. Condenser charges were used to stimulate, as described previously (Bishop and Heinbecker, 1930) at frequencies from single stimuli up to 10 per second. At first precautions were taken to keep the nerves warm, but working in a room at above 25°C. this was found to be unnecessary for the results required. When stimulating preganglionically, the ganglion itself, which is the critical structure in the pathway, was probably nearly at body temperature. In other experiments, after *in vivo* results had been recorded, the nerve was removed to an incubator at 37°C., in oxygen, and analyzed under more standard conditions for comparison with previous nerve work. Since the post-

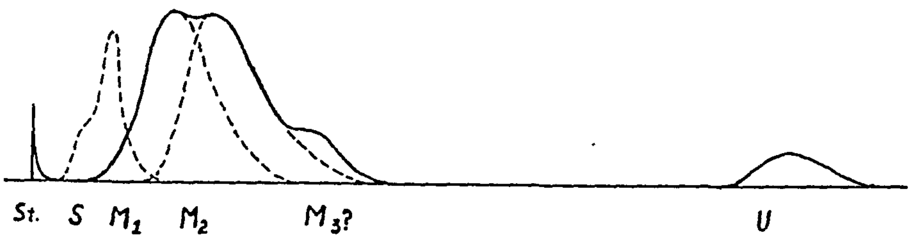

Fig. 1. Diagram of action potential waves led off from the cervical sympathetic nerve of cat or rabbit after conduction. Abscissa, time; ordinate, voltage. *St*, stimulus artefact signalling on the record the time of application of stimulus; *S*, potential wave usually present in the cat but never in the rabbit, due to a group of sensory fibers of vagus origin corresponding to those of the separate depressor nerve regularly present in the rabbit and only occasionally in the cat; M_1, potential due to first part of double group of motor sympathetic preganglionic myelinated fibers responsible for pupillary and other responses; M_2, potential of second part of this group, of slower conduction and with higher threshold, responsible for vasoconstriction, etc.; M_3, efferent sympathetic fibers of undetected function; *U*, unmyelinated preganglionic sympathetic fibers of undetected function. In the vagus, stimulation of fibers corresponding to these last two groups causes weakening of the force of the heart beat and slowing of the rhythm, respectively. Innervation of glands has not been excluded in the sympathetic.

Average conduction rates for the fastest fibers in each of these various groups, in meters per second: *S*, 25; M_1, 12; M_2, 8; M_3, 3; *U*, 1 to 0.5.

ganglionic stretch as dissected out is of somewhat greater diameter than the preganglionic, fibers of the same real irritability will have a higher measured threshold postganglionically. This corresponds with the findings of Veach and Pereira (1925), that the threshold postganglionically for retraction of the nictitating membrane of the cat was higher than preganglionically. We believe the actual fiber irritability, however, to be about the same pre- and postganglionically.

To preclude the possibility of stimulating preganglionic fibers, or ganglion cells, of low threshold, by spread of stimulus from electrodes placed beyond

the ganglion, the ganglion was tied off and tested before postganglionic stimulation was applied. Since this injury spread along the nerve, thresholds were unreliable unless the electrodes were placed as far as possible anteriorly.

In the cat, when *afferent* fibers were present in the preganglionic sympathetic, these had a low threshold and gave rise to the first potential elevation of the record (fig. 2); since the sympathetic had been severed from the vagus these fibers could give no response in the body, even reflexly. The *efferent myelinated* axons give two elevations which overlap, but can be quite sharply differentiated by threshold. The first group causes dilatation of the pupil, retraction of the nictitating membrane, and retraction of the lids by Müller's muscles in this order of increasing strength, but with very slight, and in some cases no, interval of threshold (fig. 3). The second group causes constriction of arterioles in the ear and conjunctiva,[2] and usually contraction of the pilomotor muscles between ear and eye, although in two cases we have been unable to obtain this response at all, and it is never very striking. Following this double elevation in the potential picture there appears a smaller wave, for which there was no corresponding response in the structures observed. It is not certain whether the axons involved are myelinated or not, since we have not yet obtained a nerve where such a group is isolated. For the last wave, following this, and assignable to unmyelinated axons, we have found no functional response, although it conducts into the postganglionic nerve with a synaptic delay.

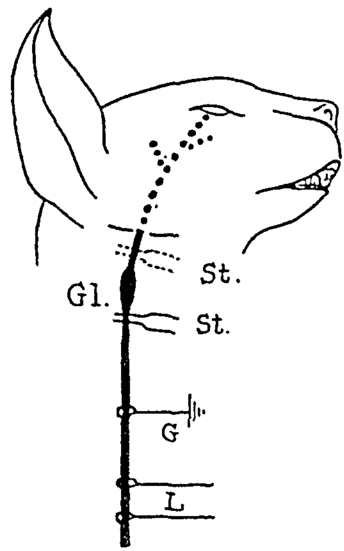

Fig. 2. Arrangement for stimulating cervical sympathetic nerve in the body, dissected out in the neck up to the bony canal of the skull, past the ganglion *Gl*. *St*, stimulating electrode, pre- or postganglionic; *G*, extra grounded electrode to prevent disturbances from the animal affecting the record led off via *L*, electrodes leading to the cathode ray oscillograph.

Stimulated postganglionically, the pupil contracts at a strength of stimulus so nearly the same as that of the corresponding preganglionic fibers that the difference in the sizes of the nerves accounts for the slight increase, and the fibers may be judged to have the

[2] By means of an ophthalmoscope we thought we could detect constriction of small vessels in the retina due to stimulation of the same fibers which caused constriction in the vessels of the conjunctiva and ear. Since the technic of these observations complicated an already elaborate experiment, further and more definitive studies of the retina have been postponed for a separate investigation.

same intrinsic irritability. A slight increase of threshold causes response of the nictitating membrane, and of Müller's muscle. To obtain blood-vessel constriction the stimulus must now be increased to at least six times the threshold for the pupillary response. This same interval is observed preganglionically between thresholds for the most irritable fibers of the myelinated and unmyelinated groups.

In the rabbit, *preganglionic* stimulation causes the same responses at the same intervals of threshold, and corresponding to the same potential components, except that pilomotor effects have not been observed. Postganglionically, no effect has been observed until stimulation is applied of a strength that activates *un*myelinated fibers. In the Belgian hare where experimentation in the body gives results identical with the rabbit, a long postganglionic stretch available for experiment enables us to check up, on

Fig. 3. Thresholds and fiber types in cervical sympathetic trunks of cat and rabbit.

ORGAN	CAT				RABBIT			
	Threshold		Fiber type		Threshold		Fiber type	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Pupil	100*	100–150	Myelin.	Myelin.	100	600–800	Myelin.	Unmyelin.
Nictitating membrane	100–150	150–250	Myelin.	Myelin.	100–150	600–800	Myelin.	Unmyelin.
Müller's muscle	100–150	150–250	Myelin.	Myelin.	100–150	600–800	Myelin.	Unmyelin.
Constrictors to blood vessels	200–350	600–800	Myelin.	Unmyelin.	200–300	600–800	Myelin.	Unmyelin.
Pilomotor muscles	300–450	600–800	Myelin.	Unmyelin.				
**	600–800	700–1,000	Unmyelin.	Unmyelin.				

* Arbitrary value.

** Action potential 1 meter per second or less with synaptic delay in ganglion; no response of animal detected.

the excised nerve, the correspondence between threshold and conduction rate, refractory period, etc., by which anatomical fiber types may be recognized physiologically. It is then confirmed that the responses to postganglionic stimulation with high threshold compared to the preganglionic, are the responses of postganglionic *un*myelinated axons only.

The cervical ganglion. Considering the whole sympathetic motor pathway from cord to eye, the most interesting difference between this and the *somatic* motor pathway lies in the synapse at the outlying ganglion. There has been some difference of opinion as to whether such a ganglion acted merely as a relay along the path to the eye, or whether it exhibited some of the functions of a central-nervous-system mechanism. Querido, for instance (1923), espoused the latter belief, while Veach and Pereira (1925) took the former view. We believe the topic can be clarified by considering some of the properties of the nerve fibers and ganglion

cells involved, together with the character of the impulses conducted through the ganglion under various conditions.

In the first place, to determine whether summation of impulses takes place in the ganglion, and whether, once stimulated, the ganglion cell gives an after-discharge like a sense organ or a nerve cell in the cord, the muscle end-organ response is not a satisfactory indicator, especially when the muscle concerned is unstriated, and does not behave in an all-or-none manner.

In the second place, the nerve fibers as well as the ganglion cells are easily depressed by rapid or strong stimulation, and even while normal show long refractory periods. We do not believe that accurately interpretable results can be obtained by stimuli more frequent than ten per second. Even at this rate the threshold does not remain constant, and fatigue of the ganglion cells sets in rapidly, and a stimulus strong enough to activate the least irritable fibers may injure the most irritable under the stimulating electrode, making accurate control of threshold impossible.

Under normal circumstances there is no treppe shown in the nerve response, pre- or postganglionically. That is, a single stimulus to the preganglionic nerve gives the same height of response when repeated, up to a rate that causes submaximal response in the fibers, or gives fatigue at the point of stimulus. This rate is not more than ten per second, and summation of stimuli in fibers for which one stimulus is just subthreshold would not be expected at 100-sigma intervals. Higher rates of stimuli such as were used by other workers have not been tested. Postganglionically, each preganglionic impulse is followed by a single discharge, or by a series of waves characteristic of the fiber types stimulated, each fiber responding only once. As a threshold-preganglionic stimulus is increased, the postganglionic response increases proportionately, both responses reaching their maxima together. There is thus no summation of the stimuli from the preganglionic nerve in the ganglion cells, no spread from one pathway to another, nor facilitation of one by the other detectable, and no after-discharge. This should be qualified by the consideration that there are many more fibers postganglionically than pre-, even in the main trunk, disregarding recurrent branches, so that it must be inferred that each in-going fiber stimulates many ganglion cells. That this is the case is further shown by the datum that wave for wave the postganglionic nerve gives as high amplitude of potentials as the preganglionic, indicating that all the fibers are being activated, since inactive fibers would shunt the active ones, and the proportion of the total active, not their absolute number, determines the potential amplitude. So far as we can see, then, the one fiber connecting with many ganglion cells activates them all at each discharge, at least for slow rates of stimulation (1 to 10 per second).

At faster rates, these events are complicated by the refractory phase

phenomena of nerve and ganglion. The absolutely refractory period after stimulation of the pre- and postganglionic fibers of the same type is the same within limits of variation of our experimental conditions, and the refractory period of the unmyelinated fiber is but slightly longer than is that of the myelinated (3.5 and 4.5 sigma, Bishop and Heinbecker, 1930). The relatively refractory periods presumably correspond, although we have not specifically plotted them. The refractory period of the ganglion cell or synapse is several times this under our experimental conditions (20 to 30 sigma), but this value may not be the true *absolutely* refractory period. The nerve responding all-or-none delivers to the synapse a fixed stimulus, which we cannot increase in an attempt to determine whether, if stimulated still more strongly, the postganglionic element could respond sooner after a first response. Now as the time between two stimuli to the preganglionic nerve, both giving maximal responses, is shortened, the postganglionic response, presumably also the all-or-none maximum for each fiber responding at this rate, falls off gradually as far as is observable, and we have not been able to determine as yet whether the falling off in total amplitude of the nerve response is due to the dropping out of a few fibers from each of the groups synapsing with single preganglionic fibers, or whether such groups fall out as units. One would expect the former, as not all synapses even with a single fiber would be expected to be exactly alike. At any rate, the refractory phases recorded are those of the fibers and cells which recover most rapidly, and such fibers at least are usually those having the lowest threshold.

Discussion. Querido (1924), and Veach and Pereira (1925) both report motor effects maintained, and even increasing in response to rates of stimulation above those employed by us. Veach specifies that at high rates the nerve shows stimulus fatigue, but he found an increase of effect, pre- or postganglionically stimulated, up to 140 per second with strong stimuli. Even at 595 and over per second, nearly the maximum contraction was obtained. It is obvious that a nerve fiber with an absolutely refractory period of 3.5 sigma cannot possibly respond to more than about one-half this higher frequency, and that a ganglion cell with a period of 20 or more sigma cannot respond to more than one-tenth; therefore, when Veach records the same response from pre- as from postganglionic stimulation, and at high rates as at low rates, it is certain that the end-organ response is not a precise criterion of the activity of the nerve or ganglion cell. The "minimum limiting rates" of Querido correspond reasonably to our refractory periods, but his "maximum limiting rates" offer the same difficulties of interpretation in terms of nerve response as do Veach's results.

But while we specify 10 per second as the maximum rate which we can safely *interpret*, this is certainly not the limit of effective muscular summation, for even though in each fiber the impulse in the relatively refractory

period of the last one is of less intensity than a first impulse, it may still affect the muscle. Whether it affects the smooth muscle *maximally*—that is, equally to a first impulse—would be an interesting speculation in the case of an end organ which apparently does not itself obey the all-or-none law. We have not attempted to sort out the influence of factors such as inhibition of the Wedenski type, stimulus fatigue, and summation at the muscle of submaximal stimuli, but have sought rather to avoid their complications in the interest of simplicity in the interpretation of the specific problem of nerve and ganglion behavior. Certainly the normal action in the body would be limited to those rates of response that permitted nearly the normal maximum activity of each element. Whether at higher rates than this the cervical ganglion can do more than simply relay impulses over a multiplied pathway we cannot say, but we doubt whether it does so in response to such impulse frequencies as normally occur in the body.

We cannot therefore confirm Querido, that "preganglionic stimuli of any frequency above the minimum critical rate sets up impulses which pass beyond the superior cervical ganglion at frequencies between 120 and 160 per second," since we find that the refractory period of the ganglion cells would limit such frequencies to about 50 per second; and we can detect no after-discharge from the ganglion; nor can we place the significance which Veach seems to assign to it, on his finding that even at high frequencies the effects of pre- and postganglionic stimulation are of the same amount, because we do not believe that nerve impulses of such frequency ever reach the muscle by way of the postganglionic fiber, or would be of significant intensity if they did.

In spite of the fact that a single impulse is conducted through the ganglion without requiring summation, it is not always true that the response to a single impulse is detectable in the peripheral motor organ. For the nictitating membrane, a single stimulus pre- or postganglionically delivered, results in a visible retraction, even when not all of the group of axons involved in this reponse are stimulated. A single maximal stimulus results in, roughly, one-half complete retraction, with slow relaxation, variable from animal to animal. Two such impulses may result in fairly complete disappearance of the membrane behind the angle of the lids. For the pupillary response, a single stimulus below maximal can be detected, especially if in decerebration the parasympathetic pathway has been broken, or if the eye is not too brightly illuminated. It usually requires, however, more than two maximal stimuli (at half-second intervals) to cause anything approaching complete dilatation. For the movement of hairs, nothing less than two maximal stimuli, or a longer series of submaximal ones, can be detected at all, and for vascular constriction, observed in the eye or ear with a low-power lens, only a series can be detected with

certainty. These differences are presumably only quantitative ones in end-organ response, the amount *observable* being the variable. Applying the criteria of Lapicque (1928) or of Fredericq (1930), certain fibers of the cervical sympathetic would fall in the class of "iterative" nerves, in that more than one stimulus is required for a "response" of the end organ. Since, however, the pre- and postganglionic fibers both respond to each stimulus, and since the end organs do not differ materially, being all of smooth muscle, except in degree, we feel that any definition so far given of an iterative nerve is without meaning, and is predicated surely on inadequate means of observation. We have no doubts that for each nerve impulse a muscular response takes place in the fibers innervated, even when a number of summated responses are required for observational threshold, or even though the first response may be expended in taking up what might be termed the "physiological slack." The idea that reiterated stimuli from nerve to end organ are required to overcome the difference in chronaxie between them, as inferred by Lapicque, may thus arise from the inadequacy of the means of recording; and we see no reason for believing that any peripheral nerve in its normal state lacks the ability to deliver a single effective stimulus to its end organ, even though in some cases the unit of response may be a small one.

The character of the summation, however, of stimuli applied to such nerves, seems to us of considerable significance. It is easy to so adjust a strength of stimulus that a few nerve fibers give a response as indicated by the oscillograph for each stimulus, but with so few fibers responding that no response of the end organ is visible. Now if either the rate of stimulation to these few fibers is increased, or if the strength is increased to stimulate more fibers of the same functional group at the initial rate, the end-organ response becomes visible, and increases progressively with increase of either rate or strength of stimulation. The important finding is that no difference can be detected in these two procedures by observation of the end response. Apparently the response is conditioned by the number of individual nerve-fiber stimuli received; that is, the number of fibers times the number of responses of each fiber, and not by either frequency or strength alone. The *threshold* for such an end-organ response is thus to be expressed in terms of number of fibers times the number of impulses per fiber; and frequency also must enter into the picture as a factor separate from mere numbers of stimuli. Threshold for nerve response on the other hand is, for physiological frequencies, merely a matter of intensity of stimulus.

The most obvious analysis of this situation would appear to be that each nerve fiber made connections with a separate corresponding group of effector elements, temporal and spatial summation of their effects amounting to the same values. This applies satisfactorily, for instance, to the

case of skeletal muscle. However, in the autonomic nervous system two complications enter in: one, the increase in number of fibers beyond the outlying ganglion, and the other, the nerve plexus in smooth muscle itself. These seem to be economical mechanisms for distributing stimuli over a wide area without the enormous number of fibers that would be necessary if all led out from the spinal cord direct. Further, in the case of dilatation of the pupil, or constriction of the vessels of the ear, weak stimulation activating few fibers repeatedly, shows no sign of punctate distribution, any stimulus being evenly effective over the entire area innervated by that fiber component. We cannot refrain from the speculation that here is a mechanism for spatial and temporal summation more like that of the central nervous system than it is like that of skeletal muscle. In the central nervous system also, the reflex threshold appears to be determined not by strength of afferent nerve stimulus alone, or by frequency alone, but more nearly by the product of the number of fibers stimulated times the frequency of stimulation.

SUMMARY AND CONCLUSIONS

The action potential components characteristic of nerve fiber groups in the cervical sympathetic nerve of the cat and rabbit have been correlated with the motor responses of head structures, by oscillographic recording from the nerve simultaneously with observation of the results of stimulation.

In the preganglionic sympathetic, a group of afferent fibers is usually present, which gives the first potential of this nerve at lowest threshold. Following this, the next fiber group to respond as the stimulus is increased consists of the small myelinated sympathetic fibers, present also in the rabbit nerve, where, however, no afferent fibers appear to be present. This sympathetic motor group is double in both animals, giving a bimodal potential wave. Still further increase of stimulus strength causes unmyelinated fibers to respond.

Postganglionically, the first part of this double myelinated motor group synapses with similar myelinated fibers, in the cat, the second part with *un*myelinated fibers. In the rabbit both parts synapse with unmyelinated fibers. The monkey is probably like the cat in this respect. This is contrary to Gaskell's dictum that postganglionic fibers are always unmyelinated in mammals.

Retraction of the nictitating membrane, dilatation of the pupil and contraction of Müller's muscles in both cat and rabbit result from stimulation of the preganglionic myelinated fiber group of lowest threshold. Constriction of blood vessels in the eye and ear, and contraction of pilomotor muscles result from stimulation of the similar preganglionic axons of somewhat higher threshold. No response has been detected to stimula-

tion of the preganglionic unmyelinated fibers synapsing in the superior cervical ganglion.

Postganglionically, in the cat, the first group of responses follows stimulation of the same order of strength as preganglionically, while in the rabbit the stimulus must be five or six times as strong postganglionically. In both animals the second group of responses follows only the stronger stimulus, characteristic of unmyelinated axons. In the Belgian hare, a longer postganglionic nerve available for experiment allows measurements of conduction rates which confirm the threshold findings.

The refractory period of the ganglion cell in the superior cervical sympathetic ganglion is of the order of 20 sigma, which normally limits the rate at which the postganglionic fiber responds. We find no spread of response from one cell to another, no after-discharge, and no summation of preganglionic impulses in the ganglion, although more fibers emerge from it than enter. This presumably means that one preganglionic fiber synapses with more than one postganglionic fiber, and normally causes all such fibers connected to it to respond in a volley. This would permit a distribution of the response to one preganglionic impulse over a wide area of the motor mechanism innervated by the postganglionic fibers connected with it, which seems to be what happens in the body.

While all the responses studied involve the activity of smooth muscle, one nerve response does not cause a visible muscular response in all structures. This apparently depends on the threshold for visual observation of the response in any given case, and we see no reason for calling the cervical sympathetic an "iterative" nerve.

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ON THE MECHANISM OF CERTAIN OVARIAN HORMONAL INFLUENCES ON THE CENTRAL NERVOUS SYSTEM[1]

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In a recent paper (1931) the authors were able to demonstrate a definite influence of certain sex hormones on the central nervous system of the rat, particularly on the upper motor neurone. This influence was indicated by an increase in the upper motor neurone's inhibitory action on Achilles reflex time during the oestrous phase of the sex cycle as well as in the later stages of pregnancy, and by a decrease in this action during the dioestrous interval and during pseudopregnancy. An experimental check appeared to verify these findings, in that spayed animals brought into induced oestrus displayed a reaction similar to that of intact animals in the corresponding phase of the cycle; administration of corpus luteum extracts, on the other hand, resulted in a reaction comparable to that evoked in dioestrus, early pregnancy, and pseudopregnancy. In brief, it was shown that "a lengthened Achilles reflex time accompanied any increase in the concentration of follicular hormone, or its extract, in the system of the animal. Conversely, shortened Achilles reflex time was shown to accompany an increase in the concentration of luteal hormone, or its extract."

Two possible explanations were tentatively advanced to account for these phenomena. Either 1, the hormonal content of the blood has a more or less selective action on the upper motor neurone, or 2, the influence at this level is mediated by nervous mechanisms, the stimuli for which probably lie in the hyperemia and distention of the tubes and cornua characteristic of the active phases of the sex and reproductive cycles. These hypotheses, it appeared, could be put to the experimental test, namely, through interruption of the nervous pathways leading from the genital system to the higher levels. The present report deals with some results obtained from experiments based on this principle.

Experimental methods and results. Ten normal adult female rats, the regularity of whose cycles had been determined by the vaginal smear method (Long and Evans, 1922), were bilaterally sympathectomized, with

[1] The authors wish to express gratitude to Prof. Lee Edward Travis, in whose laboratory this work was done.

ether anesthesia and under a dissecting microscope, from the level of the renal arteries through the pelvis, the operation involving complete excision of the sympathetic ganglionic chains and the parasympathetic pelvic nerves. Following an adequate recovery period the oestrous cycles again were checked for a period of three weeks. At the end of that time electromyographical Achilles reflex time records were taken during the oestrous phase and during the interval, records for several cycles being secured for each animal. All readings were obtained on the left Achilles reflex.[2]

Table 1 presents the Achilles reflex times in sigma for the corresponding cyclic phases. It will be seen that no lengthening of reflex time occurred coincident with the oestrous phase, and that no shortening was obtained

TABLE 1

Left Achilles tendon reflex times in sigma during oestrus and the dioestrous interval in bilaterally sympathectomized rats

ANIMAL	L.A.R.T.	STAGE	L.A.R.T.	STAGE	L.A.R.T.	STAGE	L.A.R.T.	STAGE	L.A.R.T.	STAGE	L.A.R.T.	STAGE	L.A.R.T.	STAGE	L.A.R.T.	STAGE
HL$_1$	6.8	E	6.5	D	6.1	E	6.5	D	6.5	E	6.3	E				
HR$_2$	6.3	E	6.5	D	6.6	E	6.5	D	6.3	E	6.5	D				
WL–	6.2	D	6.0	E	6.3	D	6.5	E	6.8	D	6.2	E				
WL$_1$	6.8	D	6.3	E	6.4	D	6.5	E	6.5	D	6.5	E				
WR–	6.3	D	6.5	E	6.2	D	6.6	E	6.1	D	6.3	E	6.5*	D	6.5*	E
WR$_2$	6.6	D	6.7	E	6.8	D	6.7	E	6.0	D	6.1	E				
HL–	6.6	E	6.7	D	6.2	E	6.1	D	6.5	E	6.0	D				
HR–	6.7	D	6.5	E	6.3	D	6.0	E	6.5	D	6.3	E	6.9*	D	6.8*	E
WL$_2$	6.5	D	6.0	E	6.0	D	6.5	E	6.5	D	6.5	E				
HR$_1$	6.0	D	6.0	E	6.5	D	6.2	E	6.3	D	6.2	E				

* Records taken two months following operation.
E—oestrus; D—dioestrus.

during the interval, in marked contrast to the findings previously reported for the intact animal (*loc. cit.*).

The failure to elicit fluctuating responses in the sympathectomized animal despite the continuance of a normal oestrual rhythm would appear to be evidence of a hormonal control of the higher nervous levels through a mechanism operating by means of the sympathetic system, and would argue for the interpretation that the increased activity during oestrus, or during its experimentally induced counterpart in the spayed animal, is mediated through excitatory impulses traversing the sympathetic system to the cord and thence to higher levels. Were the influence a direct one, it does not seem probable that sympathectomy would interfere materially with transmission of the impulse.

[2] The apparatus and the method for eliciting these reflexes have been fully described elsewhere (Herren and Haterius, 1931).

Data apparently conflicting with this view have recently been presented by Bacq (1931), who reported no change from the pre-operative norms with respect to spontaneous activity; the same oestrual peaks characterized the records in each instance. However, his results were based on averages of ten day intervals, and no attempt was made to correlate daily activity with the genital cycle. Daily runs of spontaneous activity are known to be extremely inconstant; they are also inconsistent, from cycle to cycle, for any given phase of the cycle. Averaging ten days' run therefore would have a tendency to "iron out" any effect to be correlated with phases of the sex cycle.

Vogt (1931) has reported that removal of the superior cervical sympathetic ganglia together with the cervical inter-ganglionic trunks inhibited the mechanical induction of pseudopregnancy. This was taken as *prima facie* evidence that a genital-pituitary nervous mechanism is operative, to some extent at least, in the Prolan B (i.e., luteinizing principle) release of the anterior pituitary, and that, under certain conditions, interruption of the nervous pathway results in failure of excitatory impulses from the genital system to reach the pituitary. This work awaits confirmation, but appears suggestive in the light of results reported here of lower abdominal sympathectomy. It was hoped that the reaction of our operated animals, following mechanical stimulation of the cervix, would shed some light on the question of a possible nervous mechanism. At the time attempts were made at inducing pseudopregnancy, several months after the reflex records had been taken, only four of the animals were found to be running regular cycles. Repeated attempts on these, however, failed to elicit any response and no interruptions of the cycles were noted. Four cases, however, are not sufficient to permit of any conclusion being drawn, more particularly since it is known (Slonaker, 1929) that cervical stimulation very often fails to bring about the expected reaction even in the normal animal. It is evident that further work is necessary to determine this question.

In connection with the present work, records were kept on the length of the sex cycle before and after operation. Our data corroborate those of Sweet and Thorp (1929), who reported abdominal sympathectomy to have no appreciable effect on the length of the oestrous cycle. Before operation our animals ran a cycle of 4.4 days, following operation a cycle of 4.3 days. All operations were verified by postmortem binocular examination.

SUMMARY

1. Animals bilaterally sympathectomized from the level of the renal arteries through the pelvis (including the pelvic nerve) do not show the correspondence between oestrus and lengthened Achilles reflex time that is exhibited by normal animals.

2. Bilateral abdominal sympathectomy does not alter the length of the normal oestrous cycle.

3. From results obtained, it seems highly probable that certain influences of the ovarian hormones on the higher nervous levels are effected through the sympathetic connections and are not in the nature of direct stimuli.

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THE INNERVATION AND FUNCTIONS OF THE NICTITATING MEMBRANE IN THE CAT

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The question of the innervation of the nictitating membrane (n.m.) in the mammal and the nature of its movements is still unsettled. In this Laboratory the n.m. of the cat has been recently used as an indicator of adrenin, sympathin, and liver secretion resulting from sympathetic stimulation (Rosenblueth and Cannon, 1932). Some preparations, especially under light dial anesthesia or after decerebration, were unstable and presented apparently spontaneous movements of the n.m., different from the frequent slight rhythmical contractions which smooth muscle commonly shows when lightly stretched. This fact suggested the advisability of attempting to determine the different conditions which will produce movements of the n.m. in the cat.

Anatomical data. The form of the n.m. has been frequently compared to a triangle, the apex being at the inner canthus and the base being the free external border. This morphological comparison is inadequate, since it neglects the prolongations of the membrane along the lids toward the external canthus. We shall speak of the membrane as crescent-shaped (see fig. 1).

Zernik (1928) described two groups of smooth muscle in the n.m. of the cat, one producing retraction, the other, protrusion. His schematic representation of this last action is not consistent with either the function or the true shape and position of the membrane.

Stibbe (1928) made a comparative anatomical study of the n.m. in amphibia, birds and mammals. He states that there appear to be two neuromuscular mechanisms for the protection of the eye: a III-nerve mechanism for the lid and a VI-nerve mechanism for the n.m. He claims that both mechanisms are present in amphibia and birds, in connection with the lower lid only in the frog, and in connection with both the lower lid and the n.m. in the bird. He denies, however, any connection of the VI nerve with either lid or n.m. in the mammal. He concludes that the n.m. of the mammal bears no relation to that of the bird.

In the bird there is no levator palpebrae superioris,—the movements of

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the upper lid depend exclusively on the movements of the eye. The lower lid, however, is freely and independently movable through the depressor palpebrae inferioris. This muscle (controlled by the III nerve), the quadratus, and the pyramidal (controlled by the VI nerve) determine the nictitations of the membrane. The quadratus is the equivalent of the retractor bulbi of the mammal and is controlled also by the VI.

In the mammal it is the upper lid that is independently movable, through the levator palpebrae superioris. Stibbe (1928) found insertions of this muscle in the n.m., but did not give them any importance. He denied that there are any other muscular connections with the n.m. in the mammal.

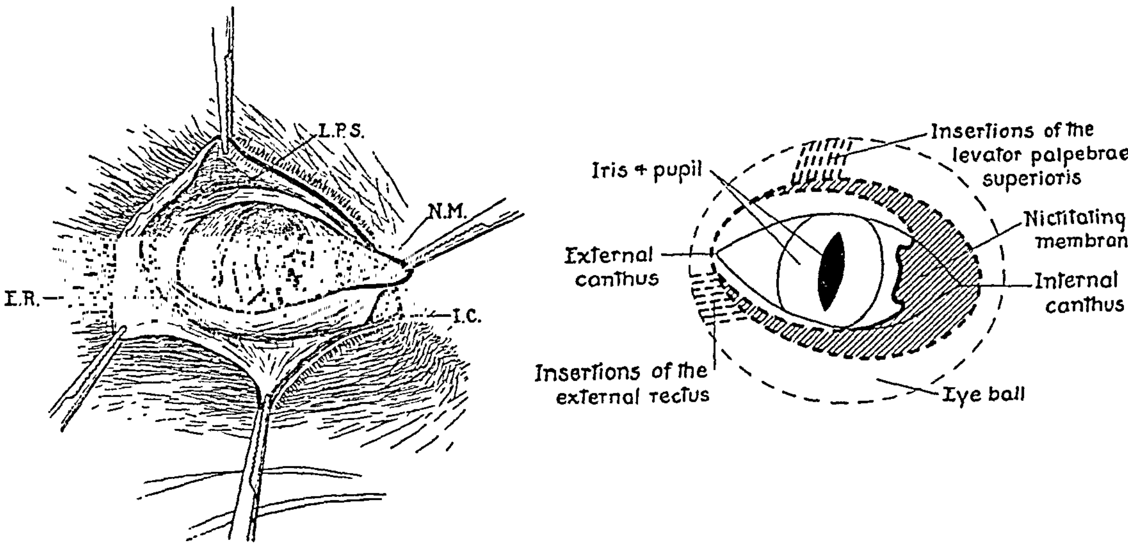


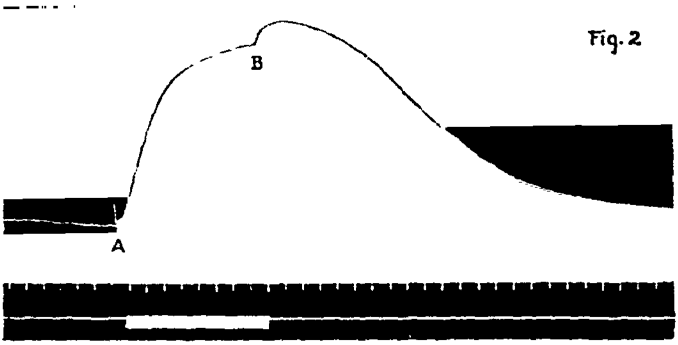
Fig. 1. Schematic representation of the shape and connections of the nictitating membrane.

Our dissections showed us that the external rectus muscle has definite connections with the n.m. The exterior fibers of the muscle do not insert in the eye, but may be traced into the inferior horn of the crescent of the n.m. We shall show that our mechanical study confirms these anatomical relations. The histological examination of the horns of the membrane, made by Mr. J. O. Pinkston, revealed the presence of striated muscle fibers, some of which may be traced to within 1 mm. of the cartilage.

The membrane, an elastic structure, is normally tense over the convex spherical globe of the eye. Being essentially a reduplication of the conjunctiva it connects with the eye and lids, and their position will influence the position of the n.m. Passive movements of the n.m. are therefore not only possible but inevitable. The problem, then, is to determine what laws govern these passive movements and whether there are any active movements which may superimpose themselves on the passive pattern.

Mechanical considerations. On retraction of the globe of the eye the n.m. will protrude, and on protrusion of the globe the membrane will retract. This is a natural consequence of the fact already mentioned, that the n.m. is an elastic sheet stretched over a smooth convex surface. The passive retraction of the n.m. on protrusion of the eyeball is illustrated in figure 2.

The passive movements of the n.m. accompanying rotations of the eye were studied shortly after death in an animal with the lids and the membrane intact. The cranium was widely opened, the brain and the superior and exterior walls of the orbit were removed. The eye muscles were pulled toward their posterior insertions and the corresponding movements of the n.m. were observed.



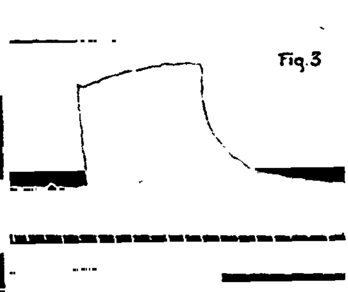


Fig. 2. Record of the retraction of the membrane on strong simultaneous stimulation of the III nerve intracranially and of the neighboring sympathetic strands. The sharp rise, *b*, in the curve, at the end of the stimulus, shows the protrusion of the eye when the retracting action of the III is released.

Fig. 3. Record of the protrusion of the nictitating membrane on stimulation of the VI nerve. The eyeball was emptied. The thread attached to the membrane was directed so that rises in the curve represent protrusion.

In pulling the muscles care was taken not to cause retraction of the eyeball. This was accomplished by preventing backward motion of the eyeball by means of an instrument. Pulling the superior and inferior recti, which causes upward and downward rotations of the eyeball, has a negligible influence on the position of the n.m. Pulling the internal rectus causes a slight retraction, the inward movement of the n.m. being less than the inward rotation of the eye. Pulling the levator palpebrae superioris causes moderate protrusion.

Pulling the external rectus produces a marked protrusion of the n.m. The movement is at first parallel to the outward rotation of the eyeball (i.e., the position of the free border of the n.m. on the eyeball does not change), but as the muscle is stretched further, the movement of the eye-

ball diminishes, whereas the membrane continues its outward course so that its free border covers a larger space between the lids.

The passive movements attending contraction of the superior, inferior and internal recti can be easily accounted for by examining figure 1. The protrusion produced by the external rectus, however, is greater than it would be if only occasioned by the outward rotation of the eyeball.

In the normal animal, when tonic sympathetic impulses to the n.m. and Müller's muscle are in play, rotation of the eye does not cause the membrane to protrude. After sympathectomy, when the membrane is paralyzed, extreme inward and outward rotations of the eye produce only the slightest retraction and protrusion of the membrane.

Active retraction due to sympathetic stimulation. Control of the membrane by the sympathetic has been thoroughly established. The fibers come from D_1 to D_4 and sometimes D_5, according to Langley (1895). According to Zernik (1928) they arise from C_8 to D_4. They synapse at the superior cervical sympathetic ganglion (Langley, 1895). They reach the bulla ossea without having come in contact with the internal carotid, According to Zernik, or running along the artery, according to Kleijn and Socin (1915). These latter authors state that the fibers pass to the base of the promontorium, enter the orbit through the vidian nerve and are afterwards not to be found in any known nerve.

Stimulation of the cervical sympathetic produces not only an active retraction of the n.m., but also a passive retraction because the eye is protruded by contraction of Müller's muscle. These two actions coöperate.

No other nerve has been shown to have a similar action on the n.m.

Active protrusion of the n.m. The existence of this movement has been debated. The early publications denied its possibility (Müller, 1872). Later Langley and Anderson (1892) affirmed the existence of an active movement since, in their observations, intracranial stimulation of the VI nerve caused protrusion of the n.m. without apparent retraction of the eyeball. Furthermore, sponging of the n.m. caused protrusion in the unanesthetized animal; this effect disappeared after the VI nerve was cut. Lewandowsky (1899) claimed that protrusion was always passive. Froehlich and Loewi (1908) confirmed Langley and Anderson's statements; they further found that curare (2 mgm.) did not abolish the protrusion, whereas it abolished outward rotation of the eye on stimulation of the VI nerve; nitrites had an effect opposite to that of curare. These authors therefore concluded that the VI contains parasympathetic fibers producing the protrusion of the n.m.

Querido (1924) again denied that protrusion is active since he did not observe movement on stimulation of the VI nerve after removing the contents of the eye. Recent authors who have used the n.m. have been

inclined to adopt Querido's view. Thus Danilewsky and Wjalkowa (1931) say that the retractor of the membrane was used in their experiments "because it has no antagonist."

Reflex protrusion of the membrane. In the normal animal mechanical stimulation of the cornea causes the n.m. to protrude rapidly and cover practically all the visible eyeball. This brusque movement may properly be called a "nictitation," notwithstanding Stibbe's assertion to the contrary. The eyeball is not seen to move in any direction.

We have studied in chronic preparations the reflex protrusion after section of one or more of the motor nerves supplying the structures within and around the orbit. In sectioning either the III, IV or VI nerves intracranially we found it advantageous to remove one entire cerebral hemisphere together with the lateral part of the diencephalon. Such cerebral ablation renders the approach to III and IV quite easy and makes it possible to elevate slightly the midbrain so as to reach the otherwise inaccessible abducens at an upper pontile level where it courses freely over the posterior fossa. The VII nerve was cut extracranially just below its emergence from the stylomastoid foramen. Five unilaterally decorticate and three unilaterally hypothalamic cats with cranial nerves intact, all in the chronic condition, served as controls; in these animals reflex protrusion of the n.m. was equal on the two sides and accordingly we were able to assure ourselves that the cerebral ablation alone does not in itself influence the reaction. Superior cervical ganglionectomy (10 cats) left the reflex intact and unmodified. In two cats III and IV of one side were cut; the reflex was unaltered on that side. The VII alone was cut in three animals and this procedure failed to alter the reflex protrusion, although reflex closure of the lids was thereafter absent. The VI alone was cut in one cat. This animal made an excellent recovery and was sacrificed after two weeks during which time reflex protrusion of the n.m. on the operated side was completely absent whereas it was normal on the other side. Postmortem examination showed without question that the abducens of the operated side had been severed. This definitely establishes the fact that reflex protrusion of the membrane involves exclusively the V and VI nerves. Curare, if given in doses sufficient to paralyze respiratory movements, abolishes the reflex.

Passive protrusion may be obtained by pressing on the eyeball, directly (if the V is cut) or through the lids. The membrane remains out as long as the pressure is maintained. The greater the pressure, the more marked the protrusion. This movement is, however, entirely different from reflex protrusion, which is not proportional to the duration or intensity of the stimulus, and which may not be accompanied by any noticeable movement of the eyeball.

It is interesting to note that whereas reflex closing of the lids is best

obtained by stimulating the internal part of the edge of the superior lid, stimulation of the cornea will usually not produce it. Stimulation of the lids, on the other hand, does not cause protrusion of the membrane. This last reflex is best obtained by stimulating the cornea, as a foreign body would, once it passes the lid barrier.

Stimulation of the cranial nerves. We stimulated the III, IV, V and VI nerves intracranially under dial anesthesia after removing one cerebral hemisphere. These stimulations were made in animals with the branches of the superior cervical ganglion degenerated, in order to eliminate a spread to them.

Electrical stimulation of the IV has no influence on the position of the membrane. Stimulation of the ophthalmic branch of the V produces variable effects which may be interpreted as reflex.

Stimulation of the III produces a moderate protrusion which can be accounted for by the retraction of the eye. This protrusion is less extensive than the one to be mentioned in the next paragraph although the retraction of the eye is much more pronounced.

Stimulation of the VI produces marked protrusion, much greater than mere outward rotation of the eye would produce. This effect persists after emptying the eyeball of its contents. In this case, however, it becomes usually less marked. This depends on the position of the n.m. before the stimulation, for, once the eye is empty, the membrane is entirely free and tends to protrude spontaneously. If, however, the n.m. is held artificially in its normal position, protrusion will occur, or the pull will be felt on the forceps holding the membrane. Querido's failure to obtain this effect under these circumstances (i.e., after the eyeball was emptied) was probably due to his not having taken the precaution of replacing the membrane in its normal position.

The type of response is very clearly that of striated muscle: rapid, occurring immediately after the stimulation begins, reaching its maximum at once, and relaxing rapidly and immediately after the stimulus ceases. These characteristics exclude the participation of smooth muscle.

It was found very difficult to record graphically the protrusion of the n.m. The nose and the malar prominences of the cat make it impossible adequately to adjust a thread so as to give a direct pull on the writing lever. Figure 3 is, however, a record of the movement on stimulation of the VI.

The response on stimulation of the VI disappears after curare is given in a dose just sufficient to abolish the respiratory movements. Froehlich and Loewi (loc. cit.) saw the protrusion of the n.m. persist after 2 mgm. of curare and concluded that it was a parasympathetic effect on smooth muscle. We believe that their dose was insufficient.

Discussion. The facts which we have reported allow us to affirm that

the external rectus muscle, controlled by the VI nerve, contains some fibers which will occasion active protrusion of the n.m., i.e., the movement is not conditioned by any attending movement of the eyeball.

This action of the VI may be exaggerated by the backward movement of the eyeball due to contraction of the retractor bulbi.

The n.m. of the mammal has the same significance as the n.m. of the bird. Its functions and innervation are similar. In both classes of animals there are two mechanisms, a III-nerve mechanism for the lid (superior in the mammal and inferior in the bird), and a VI-nerve mechanism for the n.m. The rôle of the levator palpebrae superioris, which, as we have said before, has connections with the superior horn of the membrane, is probably the same as that of the depressor palpebrae inferioris in the bird: that of fixing the lid and this part of the membrane during the protrusion.

The VI nerve contains two sets of fibers, one controlling outward rotation of the eye and the other governing the protrusion of the n.m. These functions are independent of one another. The same duality exists in the external rectus muscle, some of its fibers rotate the eye outwards and some cause protrusion of the n.m. These last muscle fibers are homologous to the pyramidal of the bird.

The n.m. presents the paradox that smooth muscle innervated by the sympathetic (the retractor mechanism) has striated muscle as an antagonist (the protrusion mechanism). The different functions of the two mechanisms are in harmony with their different histo-physiological arrangements: retraction (smooth muscle) is usually tonic and stable, whereas protrusion (striated muscle) occurs only sporadically.

The closing of the lids and the protrusion of the n.m. constitute two independent reflexes, the result of which is the protection of the eye from foreign bodies. Closure of the lids occurs when the lids themselves, especially the nasal extremity of the external edge of the superior lid, are stimulated. The afferent path of the reflex arc is the V; the efferent path is furnished by the VII. Protrusion of the n.m. occurs when the cornea is stimulated. In this case the afferent path of the reflex arc is again the V; the efferent path is supplied by the VI. Dial anesthesia may abolish the reflex protrusion of the n.m., but it does not abolish the reflex closure of the lids. The center for the V seems therefore to be active; the center of the VI (pons) may be inactivated by a sufficient dose of dial, but the center of the VII, situated below (in the medulla), remains invariably active until death occurs. Sometimes, however, if the dose of dial is slight, there occur slow pendular movements of the eye in a horizontal plane, showing relative integrity of the nucleus of the VI. It is in these cases that spontaneous contractions of the n.m. most frequently appear.

To obtain a completely denervated and inert n.m. it would be necessary

to cut the cervical sympathetic and the VI. A very simple technique suffices to cut the cervical sympathetic; but to produce a surviving cat with a severed VI is not an easy procedure. This section cannot be accomplished in the orbit without severely injuring several of the other nervous or muscular structures. An intracranial section of the nerve requires the removal of a large part of the homolateral cerebral hemisphere.

Even if the cervical sympathetic and the VI were cut there might still be present under certain anesthetics passive movements of the membrane produced by movements of the eye. It is possible, however, to obtain a perfectly inert n.m. after cervical sympathectomy, without cutting the VI. A deep anesthesia will inactivate this last nerve and the other nerves to the eye muscles. In any case, a sufficient dose of curare will remove entirely any movement due to striated muscle contraction.

SUMMARY AND CONCLUSIONS

The passive, active and reflex movements of the nictitating membrane of the cat were studied.

Passive movements exist, but are insignificant compared with the active movement.

Smooth muscle supplied by the sympathetic causes the retraction of the nictitating membrane.

Striated muscle in the external rectus innervated by the VI pair causes the active protrusion of the nictitating membrane independently of outward rotation or retraction of the eyeball.

Reflex closure of the lids and reflex protrusion of the nictitating membrane are independent processes. They have different origins and different nerve paths. They have only a similar result,—the protection of the eye from irritating agents, chiefly foreign bodies.

The nictitating membrane of the cat has the same anatomical and physiological significance as that of the bird. Their innervation is similar.

Cervical sympathectomy combined with either section of VI or deep anesthesia or curare gives a completely paralyzed nictitating membrane.

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THE ACTION OF OVARY-STIMULATING SUBSTANCE OF HUMAN URINE OF PREGNANCY ON UTERINE MOTILITY IN THE UNANESTHETIZED RABBIT

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Two to five hours before the occurrence of ovulation in the unanesthetized rabbit, the motility of the uterus profoundly diminishes from a marked type which is characteristic of oestrus, to a state approximating complete quiescence. This happens whether ovulation results from the normal stimulus of coitus or from the intravenous injection of whole human urine of pregnancy (Reynolds and Friedman, 1930a; 1930b). In view of the recent work of Friedman (1932) which shows that either growth of the Graafian follicle or ovulation and subsequent luteinization may be produced in favorable rabbits by grading the dosage of ovary-stimulating substance of pregnancy-urine, it became of interest in the present experiments to determine the changes which take place in uterine motility as a consequence of the administration of such graded, liminal amounts of the urine-substance. In addition, an attempt has been made to determine whether or not a change in motility so induced is dependent upon the presence of the ovaries or if it can occur in the absence of the tissue of the Graafian follicles and their associated structures. These experiments fall logically, then, into three groups: the uterine motility assocated with 1, doses of the urine-substance that are sub-liminal for ovulation; 2, doses that are adequate to produce ovulation, and 3, the administration of the urine-substance in suitable castrated rabbits, and hence in the absence of the ovary.

Experimental procedures. The present experiments were performed with the concentrate of human urine of pregnancy in the form of the "luteinizing hormone" of Parke, Davis & Co., to which Company we are indebted for a generous supply. As will be seen below, effects other than luteinizing were obtained with this material.

In the three groups of experiments described here, each doe was one which had recently dropped a litter. Thus one was assured of a certain degree of uniformity of the sexual state in the various rabbits. One day to a week *post partum* uterine fistulae were prepared in the several does and

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these animals were kept segregated from all other rabbits, as well as from each other, until they were sacrificed at the termination of the various experiments. Several days were allowed to elapse between the time of operation and the commencement of the experiments. The operative procedure for preparing the uterine fistula and the method of recording therefrom have been described elsewhere (Reynolds, 1930; Reynolds and Friedman, 1930a).

Only does showing good initial motility were used, since whole urine of pregnancy inhibits the spontaneous motility. In these experiments no second or third injection of the urine-substance was made except in the presence of a background of good motility against which an inhibiting effect might be demonstrated. In the first two groups of experiments, single intravenous injections of the "luteinizing hormone" were made after an initial control record of uterine motility was obtained, and subsequent records were made at suitable intervals after the injection. Exploratory laparotomies were performed as was deemed necessary in the individual experiments. Upon recovery, later records were obtained and injections made as noted. At the end of each experiment careful examination of the uterus and ovaries or sites of ovariectomy was made. None of the animals included in this study showed uterine infection, adhesions, or inflammation.

RESULTS. a. *Doses sub-liminal for ovulation.* This series is comprised of sixteen observations of the uterine response in eight rabbits. One to three series of observations were obtained in the several rabbits with the amounts of urine-substance employed varying from 0.05–0.15 cc. in the initial injection to 0.05–0.40 cc. in the second injections and 1.0 cc. in a third injection in three does.

The essential feature of the uterine response was found to be a diminution of motility in the intervals of three to four and six to eight hours after the injection. This decrease in motility occurred in all but one instance, yet it varied, from animal to animal, from a slight decrease in the amplitude of the contractions in some instances to nearly complete quiescence in others. The more profound response was seen in most of the cases. In the one rabbit which showed no decrease in motility, the extremely marked pre-injection motility nearly doubled in the frequency of the contractions by six to eight hours after the injection of the urine-substance. Others showed this only temporarily, prior to a condition of relative inactivity. This might seem paradoxical to the general inhibitory effect described above were it not for the inexplicable observation that an increase in frequency of the contractions appears to precede a cessation of orderly, rhythmic contractions. This in turn appears to be a forerunner of feeble motility whether it be faintly rhythmic, undulatory, or arhythmical. Although this observation has been described before (Reynolds, 1931a), nevertheless, by virtue of the fact that it has been a most common observation in these

experiments, the data of the present paper have made this phenomenon seem to be a more certain indication of impending uterine inactivity than before. Its possible bearing on the uterine motility described for the rat has been recently discussed elsewhere (Reynolds, 1932).

Between the injections, laparotomies were performed and the condition of the ovaries determined. Chief of the findings disclosed by laparotomy at these times was that in not one instance was ovulation seen to have occurred, nor was there any luteinization grossly discernible. The follicles attained a diameter, in some instances, of two to three millimeters, as measured by a caliper. It is worthy of note, moreover, that in *no instance* in which repetitive injections were made, did ovulation of the experimentally enlarged, cystic follicles take place, even in those instances in which 0.40 cc. or more of the urine-substance was employed. It was found that such an amount of this particular batch of material was capable of producing ovulation in another doe when a single first injection was made. Whenever ovulation or grossly discernible luteinization took place as a result of a second or third injection, the response was confined to new small follicles. These findings confirm the more extensive observations of Friedman (1932).

b. *Doses adequate for ovulation and luteinization.* Due to the fact that whole urine of pregnancy produced 1, profound uterine quiescence which continued for twenty-four to forty-eight hours, and 2, ovulation in each instance (Reynolds and Friedman, 1930b), this series of experiments has not been repeated at length in this work other than to confirm those findings for the somewhat purified urine-substance. The earlier results were amply substantiated throughout. Only one doe was an exception, in that at the end of twenty-four hours after the injection her uterus showed pre-injection motility, but this effect wore off spontaneously by the second day.

A brief generalization, which only approximately describes the results outlined in the two sections above, may be stated in the following manner:

Ovarian response	*Uterine response*
1. Abnormal follicular growth (cystic follicles) or no grossly discernible follicle growth	1. Transitory decrease in motility in 4–8 hours following injection; permanent return to pre-injection type in 24 hours
2. Ovulation and luteinization grossly discernible; possibly corpora hemorrhagica alone	2. Decrease in motility in 4–8 hours following injection with no return of motility in 24–72 hours
3. Ovulation of several follicles plus many cystic follicles from previous injections	3. Decrease in motility in 4–8 hours following injection, with a temporary return of motility in 24 hours followed by spontaneous occurrence of inactivity in 48 hours (only indicated in these experiments)

c. The action of the urine-substance on the oestrin-activated uterus of the castrated rabbit. Castration experiments were performed on eight animals with the injections being repeated in two animals. Thus ten observations were made in this series. Since uterine quiescence rapidly supervenes after castration, and since oestrin is the only substance that has yet induced rhythmic motility in uterine fistulae (Reynolds, 1931a), it was necessary to resort to oestrin (Theelin) injections to provide a background of spontaneous motility against which a motility-inhibiting effect of the urine-substance might be shown. Because of this it may not be said that these experiments were performed in the absence of ovarian influence, but only that they were done in the absence of ovarian tissue which might conceivably contribute to the effect.

The use of Theelin led to several complicating factors, but fortunately they were ones with which it was easy to reckon. First, the dosage was considered to be a factor of prime importance, especially in the light of recent work which showed that 5 rat units of oestrin per kilogram of body-weight overcomes the initial inhibition of motility that normally follows coitus in the rabbit. Further, since slightly less than 2 rat units per kilogram of body-weight is known to be about liminal for a good Theelin response in recently castrated rabbits, it was apparent that the Theelin dosage must be in this order of magnitude (Reynolds, 1931a). A second consideration concerned the time during the Theelin response that an inhibition of motility could be demonstrated with certainty. It is known that the time at which maximal activity occurs is about twenty to twenty-four hours after intravenous administration of Theelin to rabbits, and that such motility continues until thirty or more often forty hours after the first of four Theelin injections (Reynolds, 1931a). Accordingly, it was decided that the amount of Theelin to be used in the present experiments should be about 2 rat units per kilogram of body-weight, and that the urine-substance should be given as before, in a single intravenous injection, between the twenty-second and twenty-fourth hours after the first of four injections of Theelin.

The results obtained were most satisfactory considering the difficulties likely to be encountered. In six of the ten experiments a profound and nearly complete diminution of motility was seen; two others showed a distinct change as regards the frequency, and only two showed no change at all. Because of the fact that the decrease in motility was only transitory, and motility of the pre-injection type returned by ten and persisted for twenty-four hours or more after the injection of the urine-substance (forty-eight hours or more after the Theelin), it is obvious that this effect is a decrease which resembles that which takes place in the presence of the ovaries, yet which here occurred in the absence of ovarian tissue and so was not dependent upon it.

Particular attention should be called to the two exceptions in this series of experiments. The first of these had received 3.6 rat units of Theelin per

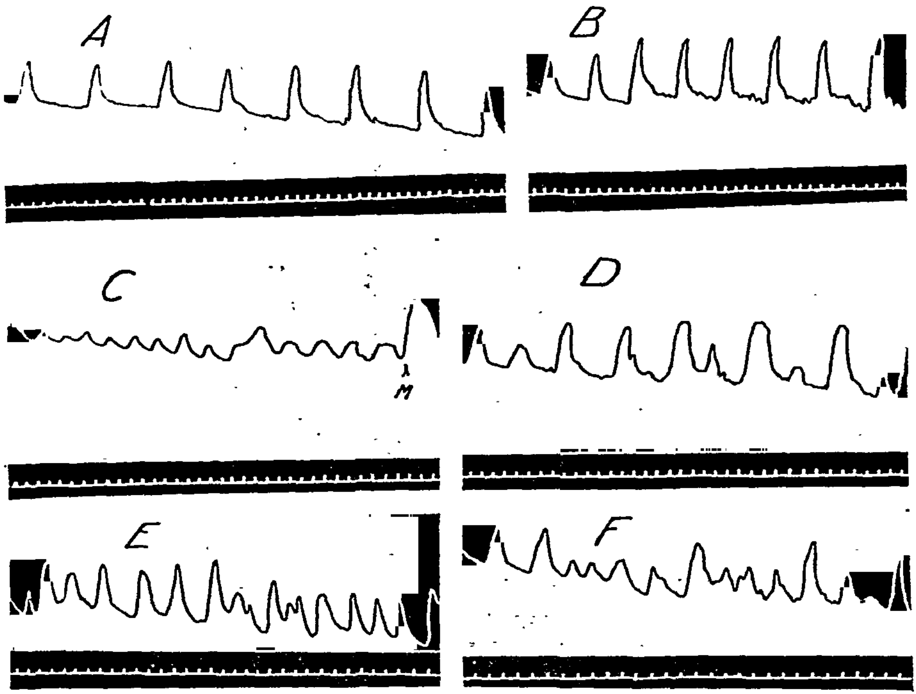

Fig. 1. Records from a Theelin-injected castrated rabbit showing that the transitory decrease in uterine motility following administration of the gonad-stimulating substance of urine of pregnancy may take place in the complete absence of ovarian tissue. Protocol of this experiment: Rabbit 15 dropped litter 11/14/31. Weight 2.60 kgm.

11/20 Castrated and fistula prepared.

11/22 1.5 r.u. Theelin per kgm. (3.9 r.u. total, or 0.0075 cc. in four divided injections of 0.0025 cc. each in seven hours; Theelin stated to contain 50 r.u. per cc.)

A 11/23 Twenty-four hours after Theelin (time of maximum response). Normal Theelin motility. Received 1.0 cc. of urine-substance, 10:30 a.m.

B 1:30 p.m.; 3 hours after urine-substance (see text for discussion of significance of the increase in rhythm).

C 5:30 p.m.; 7 hours after urine substance.

D 9:30 p.m.; 11 hours after urine-substance. Compare with pre-injection motility in A above.

E 11/24 Twenty-four hours after urine-substance (48 hours after Theelin).

F 11/25 Forty-eight hours after urine substance (72 hours after Theelin). A typical waning of the Theelin effect as described elsewhere (Reynolds 1931a).

Autopsy: No sign of uterine infection or adhesions. Sites of ovariectomy clean. Time, 10 seconds. *M*, mechanical response to indicate the potency of the system. One-fifth original size.

kilogram of body-weight. This amount is almost certainly near the threshold for a Theelin response during the first day or so of normal pseudo-pregnancy (Reynolds, 1931b). Since this doe later showed a profound uterine response resulting from an injection of urine-substance after only 2.0 rat units of Theelin per kilogram of body-weight, it is reasonably certain that this exception may be explained on the basis of too high an initial dosage of Theelin. The second exception can hardly be included in this series of experiments for it died on the sixth day following the Theelin injections from a diffuse peritonitis. Since she had diarrhea on the third day, it is quite certain that the infection was a result of the operation at which time a brown mass of necrotic fetal membranes was removed from the vagina as it was transected in the course of preparing the fistula. Inasmuch as the condition of the doe was not suspected until the experiment was well under way, however, data from her have been included in this series, yet it seems but fair to point out that she is the only doe rendered unfavorable on account of infection, and that not of the uterus.

Discussion. *Theelin-activated uteri in castrated rabbits.* A word should be said regarding the relatively large amounts (1.0–2.5 cc.) of urine-substance employed in these castration experiments. No attempt has been made to determine the minimal effective dose, but only to demonstrate whether or not the effect may be obtained without the follicular and associated apparatus. It would seem that until one first knows the quantitative amounts of oestrin normally present in a *post partum* doe, as used in these experiments, and of the various other hormones concerned in the post coital uterine quiescence, such quantitative studies would yield little to our understanding of the underlying cause of this transitory type of uterine quiescence.

Graded doses and the ovary response. The data of the above experiments completely bear out the findings of Friedman; namely, that in suitable dosage either excessive follicular growth or luteinization may occur, the latter condition being associated with ovulation or intrafollicular hemorrhage and retained ovum. They emphasize a fact pointed out by Friedman, that not only repeated injections of subliminal amounts of the urine-substance fail to elicit ovulation in the cystic follicles so produced, but that doses ordinarily adequate for ovulation are without effect on these experimentally produced cystic follicles. Chief characteristic then of the ovary-response is that the effects are regular and predictable thoughout.

Graded doses and the uterine response. In striking contrast to the regularity of the ovarian response to graded doses of the urine-substance is the irregularity of the uterine response not merely to graded doses, but to the *identical* graded doses that effected the ovarian response. The latency of the uterine response is not shortened by increasing the dosage, nor is the intensity of the response increased. Indeed it may vary rather widely

with the same dosage from animal to animal, or even in the same animal upon repetition of the injection. The lack of uniformity must end here however, for in one respect there appears to be a definitely predictable phase to the uterine response. It is associated with the absence or occurrence of ovulation. If marked motility of the uterine fistula results twenty-four to forty-eight hours or longer after the urine-substance injection, it is certain that ovulation has not taken place, whereas if feeble motility or none at all is seen, it is equally certain that ovulation has taken place.

Effect of the ovary on the uterine response. By the token, that the ovarian response may be predicted from the uterine response, it is therefore clear that the uterine response is influenced by and in part is dependent upon the changes that take place within the ovary, as a consequence of the injection of the gonad-stimulating agent of human urine of pregnancy. Moreover, the high degree of sensitivity to such small doses as 0.05 cc. of the urine-substance would make this seem all the more probable. Despite the fact, therefore, that the uterine response has been demonstrated in the oestrin-injected castrated rabbit it is impossible to exclude the ovary as a contributing factor when it is present.

The bearing of these findings to the physiology of the decrease in uterine motility that normally occurs in the rabbit after coitus is difficult to appraise at the present time. Especially is this so in view of the increasing doubt that has of late been cast upon the identity of the ovary-stimulating substance of human urine of pregnancy (see Wallen-Lawrence and van Dyke, 1931). The knowledge, however, that the uterine response is inseparable from the ovarian response after coitus and that it also occurs under the conditions here described leaves the question still open as to its physiological meaning. Investigations of the uterine motility in the hypophysectomized rabbit with appropriate substitution therapy will tend to show how correct the assumption is that the urine-substance effect on uterine motility simulates in this respect the behavior of the anterior pituitary extracts. Such investigations are now in progress in this laboratory.

SUMMARY

As little as 0.05 cc. of the ovary-stimulating substance of human urine of pregnancy induces a transitory decrease in the motility of the uterus of the unanesthetized rabbit, in six to eight hours. When the ovary is present only excessive follicular growth results from such a small dosage of the urine-substance. Larger amounts (0.4–1.0 cc.) in the presence of the ovary induce lasting uterine quiescence and at the same time, ovulation. With such amounts, a similar response has been demonstrated in suitable castrated rabbits. One may conclude that the effect of the urine-substance is independent of the ovary, yet when this organ is present it probably contributes to the effect.

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THE EXCRETION OF OESTRIN BY WOMEN

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For some time in this laboratory we have been testing the urines of women for the excretion of oestrin. The first of these tests, made on the urines of pregnant women, has been reported previously (1). These women were being treated for threatened miscarriage by an extract of pregnant urine supplied to us by Parke, Davis & Company and at that time called by them "luteinizing hormone." In the eight cases reported we demonstrated a drop in the level of oestrin in the blood and urine following a course of injections. Chart 1 shows that this is probably due at least in part to increased excretion. We have since done several more such experiments on cases of threatened miscarriage which have confirmed our previous findings and have offered rather promising clinical results. Whether the effect of this hormone is direct or through the pituitary or ovary cannot at present be concluded.

We have also tested a great many urines of patients who were receiving oestrin in one form or another for the treatment of sterility or amenorrhea. In practically all of these urines there was no trace of oestrin, although the women were taking as much as 200 to 400 R.U. daily by mouth. Frank, in his extensive work on this subject, has reported the same finding (2), as has also Zondek (3).

The clue to this situation was suggested when we discovered that progestin (prepared according to the method of Corner and Allen, 4) causes the excretion of injected oestrin in rabbits (5). In the report of this work we mentioned the possibility of evolving a test for corpus luteum, and hence presumably follicular activity in women, on the basis of this finding. Such a test would be of diagnostic value in cases of sterility and amenorrhea. If in humans, as in rabbits, progestin causes the excretion of oestrin, it should be possible to differentiate between a persistent corpus luteum, regular cyclic activity of the corpus luteum, and irregular appearance or total absence of the corpus luteum. If we can assume that a cyclic activity of the corpus luteum is preceded by a ruptured follicle, the results could also be considered as indicative of the occurrence of ovulation.

The amount of oestrin normally excreted by women at any time during the menstrual cycle is so small that it can be found only by extracting large amounts of urine. Frank has found urine tests so unsatisfactory that he has used blood almost exclusively in following the cyclic level of the oestrous hormone (6). He does report, however, having found by his method of extraction a peak in the excretion of oestrin between the 17th and 24th day of the cycle (2). Zondek also reports that the excretion is tripled in the premenstrual period (3). It is at this time that the corpus luteum is presumably present. Our own results are in agreement with these findings. By our method of testing the unextracted urines, however, excretion is never demonstrated in non-pregnant women except when oestrin is being

CHART 1

Showing the effect of "luteinizing hormone" upon the level of oestrin in a pregnant woman

Mrs. B., 4 months, 5 cc. "luteinizing hormone" daily from 5/3/31 through 5/16/31.

	5/3/31 A.M. BEFORE FIRST INJECTION	5/4/31 A.M. AFTER FIRST INJECTION	5/11/31 AFTER 1 WEEK OF INJECTIONS	5/13/31 AFTER 9 DAYS OF INJECTIONS	5/18/31 2 DAYS AFTER LAST INJECTION	5/27/31 10 DAYS AFTER LAST INJECTION
Urinary excretion of oestrin						
*1 mgm. C.	−	++				
3 mgm. C.	±	+++	−			
5 mgm. C.	++		−	−		
10 mgm. C.	+++			−	±	+++
Oestrin in the blood						
6 cc. serum	+++	+	−	−		

* Amounts of urine equivalent to 1 mgm. creatinine.

administered. Frank also reports that occasionally a second peak in excretion occurs during the first few days of the menstrual flow. We have not found this in any of the cases that we have followed, although, as will be reported below, the excretion sometimes continues from the 17th day of one cycle through the third or fourth day of the next, indicating to us a delayed regression of the corpus luteum.

Our procedure is to follow the morning urines of women twice a week over a period of one or preferably two months while they are taking 200 to 300 R.U. of oestrin daily by mouth. Most of the cases reported have been taking this in the form of Progynon tablets (supplied by the Schering Corporation of New York). We have also used a chloroform extract of the urine of pregnancy made up in this laboratory. The extract is evaporated to dryness, taken up in olive oil and put up in capsules containing

about 200 R.U. each. In one experiment a normal male took 1000 R.U. of this preparation and excreted no demonstrable amount of oestrin during the following four days. This same amount has been taken repeatedly by a normal female, with consistently negative excretion, according to our method of testing, except at the time in the cycle when her ovaries would be expected to contain an active corpus luteum.

The Allen-Doisy rat smear method is used in testing for oestrin, and the urines are acidified but not extracted. Creatinine is first determined on each specimen by the simple Folin colorimetric method (6). The amount of urine equivalent to 10 mgm. of creatinine is then calculated and this amount is given to two or more spayed rats in three injections four hours apart. The smears are read 48 hours from the first injection. A full oestrous smear is indicated by $+++$; a full preoestrous smear, i.e., nucleated cells only, is called $++$; a smear that is mostly nucleated cells is $+$; and a smear showing some nucleated cells among the white blood cells is $\pm$. Only complete dioestrous smears, i.e., white blood cells only, are designated $-$. It is important to have some standard amount of urine for injection which depends upon the concentration of the urine such as the creatinine content. Injecting a given volume of urine for each test would only lead to confusing results, for even morning urines differ greatly in concentration and we have found that the total 24-hour excretion of oestrin is the same whether the urine output is 4000 or 800 cc. We use creatinine as a gauge of concentration merely because it is fully as dependable and more convenient than collecting 24-hour urines. Since a normal 150-pound female excretes about 1 gram of creatinine in twenty-four hours, 1/100th of a mixed 24-hour specimen would be approximately the same volume as the amount equivalent to 10 mgm. of creatinine.

A summary of the results on the cases followed is given in chart 2.[1] Case 1 is a patient being treated for sterility who has regular but scanty periods, lasting only one or two days. Considering the fact that she showed excretion of oestrin from the 17th to the 28th day of each month, we would infer that there is a cyclic appearance of a corpus luteum, probably preceded by an ovulation. There is another point worth bringing out in connection with this patient. During one month, not reported in the chart, her urines were followed with consistently negative results for the excretion of oestrin. It was afterwards found that her supply of Progynon had given out and she was taking none during the period between the 15th and 28th day of the cycle. This, together with like experiments on case 7, our normal control, gives rather conclusive evidence that oestrin in the forms given is absorbed when orally administered.

[1] A preliminary report of some of these cases was included in a paper read in November, 1931, before the Obstetrical Society of Boston by Dr. John Rock of the Free Hospital for Women, Brookline.

Case 2 has had no periods for three years. She was taking Progynon for six months before the routine test was started and of the urines tested during

CHART 2

Variations in the excretion of oestrin by women taking 200–300 R.U. daily by mouth

Urines tested in amounts equivalent to 10 mgm. creatinine

CASE	CONDITION	1ST DAY	4TH DAY	7TH DAY	11TH DAY	14TH DAY	17TH DAY	21ST DAY	24TH DAY	28TH DAY	4TH DAY	7TH DAY
1	Sterility and scanty periods					−	++		++	cta. +	−	−
			−	−	−	−	++		+++	cta. ±	−	−
2	Complete amenorrhea for 3 years	−	−	−	−	−	−	−	+++	++	+	−
			+	−	−	+++	++	±	−	−		
3	Sterility, regular cta.				−	−	−	+++	++	cta.	cta. −	−
			−	−	−		−	+	+++	cta. +++	cta. +++	−
4	Complete amenorrhea; hot flushes					−	−	±	+++	+++		
5	Complete amenorrhea for 3 years since birth of child	−		−	−		−		±	±	−	
6	Complete amenorrhea for 2 years since miscarriage	−		−		−		−		−		
7	Normal; regular cta.			−	−	−	+++	++	±	cta. −	cta. −	+
		−	−	−	−	−	+++	+++	+++	cta. scanty ++	cta. scanty +	±
8	Sterility				+++	+++	+++	+++	+++	+++	+++	
				+++	+++	2 weeks overdue. Stopped Progynon						

that period all but one were completely negative. From the results given in the chart, however, it would appear that corpus luteum activity occurred twice during the two months reported, but not at regular intervals.

Case 3 is a patient who has regular periods lasting five to six days. She is being observed for sterility. From the results we would conclude that her corpus luteum formation (probably preceded by ovulation) occurs regularly.

Case 4 is completely amenorrheic. She also suffers from hot flushes, which are controlled by oestrin. Her urines were followed only for a short time but long enough to indicate that she had an active corpus luteum. Since two of her urines were completely negative it seems safe to assume that it is not a persistent corpus luteum.

Case 5 has been completely amenorrheic for three years, following pregnancy. She was being given Progynon for hot flushes. From the results we would assume that she had no corpus luteum during the month that her urines were analyzed, and therefore probably no ovulation.

Case 6 has had no periods since a pregnancy two years ago which was miscarried. She also gave consistently negative excretion, indicating the absence of any corpus luteum.

Case 7 is a normal control, age 30, who has regular periods lasting six days. Her excretion would indicate that an active corpus luteum appears on the 17th day of each cycle. During the first month this lasted only to 24th day and was followed by a normally profuse catamenia. During the second month, however, the excretion continued through the fourth day of menstruation. It is interesting that the flow during this period was markedly more scanty than usual. Inasmuch as case 3 showed the same sort of thing, the possibility must be considered that the continued administration of oestrin in large amounts may in some way cause a prolongation of the activity of the corpus luteum, although we know of no theoretical basis for such an effect.

Number 8 is a case of sterility. Her periods usually occur regularly but are occasionally one to three weeks late. Six days before the test was started she had finished a normal five-day menstruation. Her urines were followed for a month and were all markedly positive for the excretion of oestrin. Considering the fact that her period was two weeks overdue, we advised her to stop taking the Progynon, since there was a possibility that she was pregnant. An Ascheim-Zondek test on her last urine, however, was negative. We would therefore conclude that she had a persistent corpus luteum.[2]

These tests appear to be of promising diagnostic value and are being continued in this laboratory with that idea in view. At present, however,

[2] Further developments on this case since this paper went to press are worth reporting. A second Ascheim-Zondek test was done when the patient was four weeks overdue, and was questionably positive. An Allen-Doisy test on this same urine showed evidence of some excretion of oestrin but not as marked as when she was taking Progynon. Two weeks later, when she was six weeks overdue, a third

they are principally interesting to us because they confirm our previous findings in rabbits (5) and indicate that in women also the corpus luteum is concerned with the excretion of oestrin.

SUMMARY

Oestrin has been given orally to women, whose urines were followed for its excretion over a period of one or two months. Eight cases are reported, including four amenorrheic patients, three sterile, and one normal control. The results would indicate that in women as in rabbits oestrin is excreted only when the organism has been exposed to the action of corpus luteum. It appears possible by this means to differentiate between a persistent corpus luteum, irregular appearance or total absence of corpus luteum, and cyclic activity of the corpus luteum.

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Ascheim-Zondek test gave a typically positive result. It is generally accepted that the urine of a pregnant patient gives a positive Ascheim-Zondek test as early as one week after a missed period and we have even found it two days after. It therefore seems probable that this patient was not pregnant at the time of the test for corpus luteum activity and while she was taking Progynon.

REFLEX SALIVARY RESPONSE IN DEHYDRATION

(Limited Water Intake, Hyperthyroidism)

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It has been reported by Kunde (1927) that dogs made toxic by the ingestion of thyroid extract or thyroxin develop a polydipsia and a polyuria, and that with the establishment of these conditions, one may at times observe excessive quantities of saliva dripping from their mouths. The polydipsia and the polyuria may reach five to six times the normal (higher values were obtained by Kunde—1927). If the quantity of water consumed by a dog in twenty-four hours can be taken as the index of the thirst which the animal is experiencing, then the study of hyperthyroid polydipsia resolves itself into the study of a type of pathological thirst. Should the drooling of saliva from the mouth bear any relationship to experimental hyperthyroidism and polydipsia in the dog, then a quantitative study of salivary secretion in both normal and hyperthyroid conditions should yield valuable information on the thirst mechanism. According to Cannon (1919), Pack (1923) and others, thirst is a sensation of the dryness of the mouth and pharynx, brought about by a diminished salivary output. On this theory, polydipsia and normal or excessive salivation should not be present at the same time.

Method. The work was done on dogs with permanent salivary fistulae of the submaxillary ducts. Details of the operation are unnecessary since the method and technique are outlined by Kleitman and Crisler (1927). The arrangement for collecting the saliva is also described by these authors. The time allowed for healing was from a week to ten days. The dogs were then trained to stand in the type of holder commonly used for the collection of gastric juice in gastric pouch dogs. A week of training was sufficient to have the dogs become accustomed to their new duties. Salivary flow was stimulated by feeding the dogs a teaspoonful of pulverized dog biscuit every three minutes for one hour with readings recorded immediately preceding each administration. Water balance studies were conducted on two of the dogs. Determinations of water intake and urine

[1] This work was supported by a grant from the Rockefeller Foundation to the University of Chicago.

output were recorded every twenty-four hours throughout the entire course of the experiment. Standard weighed maintenance diets consisting of meat (ground beef lung, and ground beef heart), bread, bone meal, and water were given to all dogs throughout the entire work. Bone meal was found to be useful in maintaining well formed stools. A series of control experiments was made prior to the thyroid feeding period. At the completion of the control period the dogs were made hyperthyroid by means of desiccated thyroid. Dogs are relatively refractory to thyroid treatment (Carlson—1912). Hence a dose of one gram of desiccated thyroid per kilogram body weight was given daily. Since basal metabolic studies are the "most specific criterion of hyperthyroidism available at the present time" (Kunde—1927), it appeared advisable to run a series of such tests on one of the dogs after the method of Kunde (1924). Temperatures and pulse rates were also recorded on three dogs but the degree of fluctuation of the pulse rate at each reading was far too great to have any physiological importance. Since the slightest physical or emotional upset produces great variations in the pulse rate in dogs (Carlson, 1912), this procedure was soon discontinued in two animals but was continued in the third in as accurate a manner as laboratory conditions would permit, since this dog was utilized for basal metabolism studies. In this animal, records of body temperature and number of respirations per minute were also recorded. Throughout the hyperthyroid phase of the research, quantitative studies of salivary secretion were conducted in the same manner as obtained under normal conditions. When the desired degree of experimental hyperthyroidism was obtained, thyroid treatment was stopped and the animals were permitted to return to normal and control salivary tests again made.

On the basis of the results obtained in the hyperthyroid experiments, it seemed important to determine more accurately the relation between tissue dehydration and salivary output. This phase of the problem was carried out on four dogs. Salivary response was determined on varying fluid intakes, including the water in the food. Two dogs were daily given a quantity of water equal to their average normal intake as determined previously by water balance studies, plus one gram of desiccated thyroid per kilogram body weight. In two other dogs dehydration was produced by the gradual reduction in the quantity of water given daily and the effects on salivary secretion were noted. Urine volumes were recorded on all dogs.

Results. The results are presented in tabulated form. It will be seen from table 1 that on unlimited water intake there is no appreciable change in the reflex response of the salivary glands in hyperthyroidism.

Since the results obtained from water balance studies on all dogs were in agreement, table 2 presents the data from one dog. Each figure represents

the average for five days. During the hyperthyroid period the polydipsia and the polyuria are well illustrated.

TABLE 1

Salivary secretion—unlimited water intake

DOG NUMBER	TOTAL SECRETION PER HOUR											
	First control				Hyperthyroidism				Second control			
	Number of observations	High	Low	Av.*	Number of observations	High	Low	Av.*	Number of observations	High	Low	Av.*
		cc.	cc.	cc.		cc.	cc.	cc.		cc.	cc.	cc.
I	8	46.5	38.0	43.1	21	53.3	30.0	41.1	8	52.2	41.6	46.3
II	8	48.1	27.2	36.5	19	54.4	34.2	43.3	7	52.5	38.0	48.1
III	8	46.7	35.0	39.9	10	56.5	35.8	43.7	5	48.5	37.2	43.6
IV	9	42.5	28.7	36.8	13	38.5	27.0	33.4	6	46.5	34.1	38.0

* Average for total number of observations.

TABLE 2

Average daily water intake and urine volume for five day periods

Dog II

CONTROL PERIOD		HYPERTHYROID PERIOD (1 GRAM PER KILO)		THYROID STOPPED	
Water intake	Urine output	Water intake	Urine output	Water intake	Urine output
160	151	420	197	754	392
319	188	568	465	454	278
161	218	530	356	492	263
112	177	436	355	360	272
		522	421	380	264
		602	540	352	256
		533	392	425	275
		685	594		
		870	700		
		1,207	618		
		930	669		
		1,001	678		
		814	602		
		910	752		
		873	460		
		1,014	604		
		1,192	726		
		1,100	905		

Part of the data on salivary response in dehydration are presented in table 3. With the water intake kept constant, the ingestion of desiccated thyroid induced decreased salivary response, the animals showing at the

same time symptoms of dehydration. Thyroid was continued but the dogs given water ad libitum. Salivary response returned to normal within twenty-four hours. The experiment was repeated with the same results.

The voluntary daily water intake of dog IX was 400 cc. Reducing the water intake to 300 cc. per day, caused no appreciable change in salivary response. However, with a reduction to 250 cc. symptoms of dehydration (restlessness, hyperemia of oral and pharyngeal mucosa, dry nose and eyes, difficulty in swallowing) appeared, but salivary response was only slightly decreased. Further reduction to 200 cc. of water caused severe dehydration with an appreciable reduction in salivary response. With the restoration of 400 cc. of water daily, the salivary output returned to normal.

TABLE 3

Salivary response per hour. Dehydration

Dog VII

CONTROL—400 CC. WATER INTAKE	400 CC. WATER INTAKE PLUS 1 GRAM THYROID PER KILO	1 GRAM OF THYROID PER KILO PLUS WATER AD LIB.
cc.	*cc.*	*cc.*
47.6	42.4	48.2
44.1	35.8	49.5
43.6	32.7	50.3
47.9	33.7	44.5
42.5	29.5	43.5
	18.2	
Av.......45.1	Av.......32.0	Av.......47.5

Dog IX

FIRST CONTROL—400 CC. WATER INTAKE	300 CC. WATER INTAKE. DAILY EXPERIMENTS	250 CC. WATER INTAKE. DAILY EXPERIMENTS	200 CC. WATER INTAKE. DAILY EXPERIMENTS	SECOND CONTROL—400 CC. WATER INTAKE
cc.	*cc.*	*cc.*	*cc.*	*cc.*
32.0	35.3	32.0	19.5	34.3
31.1	44.8	27.5	21.7	27.5
32.7	27.2	29.2	17.5	32.2
36.8	24.8	29.0	21.5	32.1
32.8	30.2	28.6	18.3	32.4
Av..33.1	Av..32.3	Av..29.2	Av..19.7	Av..31.7

Discussion. If salivary secretion regulates thirst, then one should expect an alteration in the reflex response during hyperthyroidism, because of the associated polydipsia as illustrated in table 2. This was not found. The response of the salivary glands remained normal despite the increased water intake. Further, Doctor Montgomery (1931) found no increase in the water intake in dogs whose salivary glands were extirpated, that is, in dogs with relatively dry mouths.

Table 3 illustrates that with controlled water intake thyroid causes dehydration. Dehydration was also produced by the gradual reduction in water intake. Throughout the entire phase of this work, symptoms of dehydration presented themselves before any reduction in salivary response.

The daily volume of urine varied directly with the salivary response in the experiment of table 3.

CONCLUSION

1. The reflex response of the submaxillary gland does not change in hyperthyroid polydipsia.
2. When dogs are kept on the water intakes voluntarily consumed prior to thyroid feeding, desiccated thyroid produces dehydration and decreased salivary response.
3. Dehydration symptoms appear before any appreciable diminution in the reflex response of the salivary glands. Salivary response is, therefore, not a delicate measure of tissue dehydration, at least in the dog.

The author wishes to express his appreciation for the aid and advice given him by Dr. A. J. Carlson, under whose supervision the work was carried out.

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NERVE-MUSCLE CHRONAXIE MEASUREMENTS AND THE PHI GAMMA CURVE[1]

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A detailed survey of the data obtained from chronaxie measurement on the nerve-muscle group has shown that the values typically exhibit considerable variability (Fredericq, 1928). This fact makes any interpretation of the chronaxie relationships between various muscle and nerve groups extremely untrustworthy, when the investigator must resort to a selection of the best values, or the apparently most reliable cases. Preliminary experiments on the human subject showed a marked variability from one chronaxie determination to the next, which not only made impossible any conclusions concerning the relationship between muscle-groups, but also necessitated the study of the nature, extent and course of the variability itself if any experiment on muscle-group relationships were to be considered significant.

Variability in sensory thresholds has received considerable treatment by Urban (1909; 1910), Culler (1927), Boring (1916) and others. Maximum reliability in selecting a threshold value in these psychophysical procedures is obtained by statistical treatment of the data derived from the constant process method. This method consists essentially of selecting a range of stimulus values, the upper limit of which always produces a response and the lower limit of which never produces a response. This range is divided into equal steps and 100 stimuli applied on each step. The results of this method when plotted give the constant process ogive (Phi Gamma curve). Since thresholds of motor excitability in the nerve-muscle complex apparently exhibited a similar variability, the Constant Process Method was applied to determine the most probable threshold. In addition the statistical methods available for the measure of sensory threshold variability were applied to motor excitability.

Chronaxie measurements were taken on man by the usual technique of condenser discharges described by Bourguinon (1923). The apparatus differed from that of Bourguinon in that the electrodes for the motor points

[1] The experimental work outlined in the present paper was completed at the University of Iowa.

of the muscles were fastened securely in the proper position after the motor point for a given muscle had been located. In addition a highly sensitive optical lever was devised to facilitate the observation of the threshold twitches.

The results showed that a single threshold determination gave a range of chronaxie values. At the upper limit of this range 98 to 100 per cent of the stimuli produced observable responses while at the lower limit from 2 to 5 per cent of the stimuli produced observable responses. The percentage of responses observed at each of from five to seven equal steps in this range distributed themselves according to the constant process

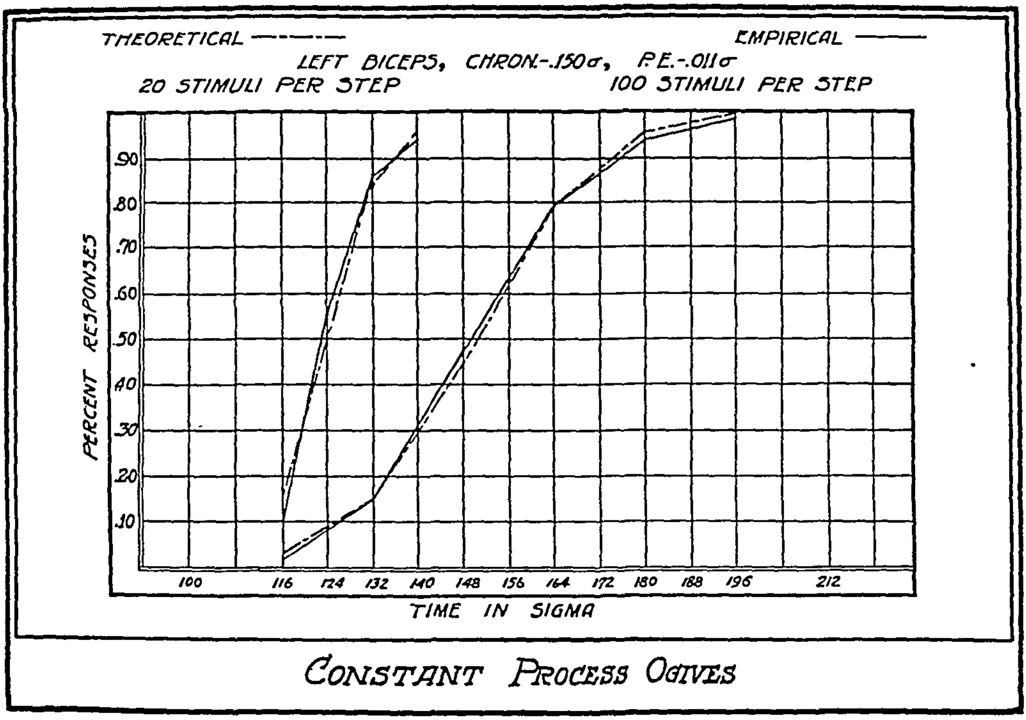

Fig. 1

ogive (the Phi Gamma curve, see fig. 1).[2] The empirical curves obtained from applying 100 stimuli at each step and in other cases by applying only 20 stimuli at each step were found (by the Chi squared test of goodness of fit, which is the mathematical procedure to determine the extent to which the empirical or experimental curve for a given threshold fits the theoretical Phi Gamma curve (Kelley, 1924)) to fit the theoretical Normal Curves

[2] The constant process ogive or the Phi Gamma curve is the ogive shaped curve depicting the distribution of responses which occur on determination of any psychophysical threshold (light, sound, cutaneous sensitivity to pain, pressure and so on) by the constant process method. The threshold in other words is not sharply defined, but is graded according to an accumulative chance distribution over a considerable range of stimulus values (Thurstone, 1927; 1928).

very nearly to perfection.[3] (See fig. 1.) This fact makes the use of the complete constant process method an accurate and convenient means of determining chronaxie thresholds and their variabilities.

Further results showed that in taking chronaxie measurements on man (and likewise to a lesser degree on the intact frog) it was advisable to apply only 20 stimuli to each of five steps in the range of chronaxie values. A study of the duration of the chronaxie range demonstrated that the range for the determination by the constant process method shifts in value before 100 stimuli can be applied to each step, but will ordinarily remain constant for 20 stimuli per step. It is significant that the curves obtained from administration of 20 stimuli on each step are practically as good fits as those obtained with 100 on each step (see fig. 1). The fact of a shifting range made the measurement of a single definite chronaxie extremely difficult at times, and further, made it impossible to determine a legitimate chronaxie value without the use of the constant process method. These shifts were probably due to the phenomena of subordination described by L. Lapicque (1923; 1928) and will be mentioned more in detail in a later section in order to demonstrate their basic physiological importance.

The procedure for determining a chronaxie value by the constant process method may be summarized as follows:

1. Determine by a few preliminary stimuli the value of stimulation (at double the rheobasic intensity) which always produces a response. This is the upper limit.

2. In like manner determine the time value just a step below the lowest value at which a response can be observed. This is the lower limit.

3. Divide the range between the upper and lower limits into five equal steps.

4. Beginning with the lower limit give five stimuli on each step up to the upper limit, recording the number of responses observed at each step. In like manner proceed from the upper limit down and repeat the process until a minimum of 20 stimuli has been given at each step. (For perfect accuracy 100 stimuli should be given at each step. The reason for selecting 20 is explained above.) It is necessary to check the rheobase after 20 stimuli or even more often at times to avoid spurious changes in chronaxie.

5. When the percentage of responses observed at each step is recorded, the chronaxie may be determined by the formula for the constant process.

$$M = \frac{(\text{Sum } xW)h - (\gamma W)}{(\text{Sum } W)\ h}\ (c.i.) \quad \text{Guessed Mean}$$

[3] One of the authors (Jasper) has recently obtained quite similar curves by the application of Lapicque's electrodes directly to the sciatic nerve of the frog while studying in Lapicque's laboratory at the Sorbonne on a grant from the Rockefeller Foundation.

Where $c.i.$ = no. of units between each step
and x = deviations from the guessed mean
W = Urban's weights for the constant process (*Arch. f.d. ges. Psych.*, 1909)

$$h = \frac{(\text{Sum } W)\ (\text{Sum } x\gamma W) - (\text{Sum}\gamma W)\ (\text{Sum } xW)}{(\text{Sum } W)\ (\text{Sum } x^2 W) - (\text{Sum } xW)^2}$$

6. The probable error for a given chronaxie may be determined from the formula (Culler)

$$P.E. = \frac{0.84535}{h\sqrt{2 \text{ Sum } n\ w\ p}}$$

Where n = number of stimuli on each step
W = Urban's weights
p = per cent responses observed on each step.

One experiment will serve to illustrate the use of this method and some of its advantages. Electrodes were applied to both the right and left biceps of subject II so that determinations could be made on each muscle in rapid alternation merely by the shifting poles on a double throw switch. One hundred stimuli were appled first to the right and then to the left biceps (20 on each of five steps) alternately until 500 stimuli had been applied to each muscle. This series was completed in 1 hour and 10 minutes. The chronaxie of the right biceps was found (by the Constant Process) to be 0.076 sigma with a S.D. of 0.009 sigma. The chronaxie of the left biceps was 0.150 sigma with a S.D. of 0.017 sigma. Since the obtained difference of 0.074 was four times its S.D. (0.019) it can be considered statistically significant.

In taking chronaxie measures in many physiological experiments it is somewhat impractical to use the full constant process for the determination of the threshold. If the formula is not used the most accurate threshold is that which gives responses 50 per cent of the time. (Use of the formula gives the more accurate threshold value relative to the actual data obtained, and presupposes a threshold at fifty per cent of responses in the ideal case.) In no case can an observed difference between two chronaxies be considered statistically significant if it is less than 15 per cent of the higher obtained value (the percentage equivalent of 3 P.E.). If the significance of the differences is calculated one may find differences which are reliable at a lower percentage but they can not be safely assumed without calculation.

It was found by use of the constant process on a continuous series of thresholds (giving 20 stimuli on each step for each threshold) that the chronaxie in a given muscle varies in a cyclic course throughout a six or seven hour period. Taking subject II again, as an example, the chronaxie at the crest of the cycle on the left biceps was 0.221 sigma (S.D. 0.007).

At the trough of the cycle the chronaxie was 0.124 sigma (S.D. 0.008). The difference between the chronaxie at the crest and at the trough of the cycle was nine times its S.D. indicating a decidedly significant difference between crest and trough values. This particular cycle was approximately 2 hours from crest to crest with the trough falling 68 minutes from the first crest. Many other cycles have been recorded which vary in time from 40 to 130 minutes from crest to crest.

Individual differences in extent, in time, and in regularity or smoothness of the cycles were observed as well as differences in the same individual from day to day. Numerous checks made it seem highly improbable that the cyclic variations could have been caused by polarization. Rheobase and chronaxie usually vary together and usually the chronaxie variation in bilaterally paired muscles follows approximately synchronous cycles. The extent of the variation within the cycle is always much less for the muscles on one side of the body than for those on the other. Some chronaxie curves which appear rough and uncyclic upon first observation smooth out into definite cycles when the quantity curve (CV) is plotted instead of the chronaxie curve, the latter being affected reciprocally by variations in the rheobase. The curves of chronaxie variation were not considered cyclic when the rheobase values varied inversely with the chronaxie values.

SUMMARY

The constant process method and its statistical procedures were shown to be applicable to the determination of the time threshold of excitability. A study of the variability of chronaxie values showed them to be cyclic in course. Since peripheral chronaxies have been shown to be very closely related to the activity of the entire nervous system (chronaxie of subordination) the cycles observed may be of basic physiological and psychological significance.

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RECOVERY OF THE TONGUE FROM CURARE PARALYSIS, FOLLOWING PROLONGED STIMULATION OF THE HYPOGLOSSAL NERVE

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In a preliminary report (Boyd, 1931) some of the work dealt with in this paper has already been summarized. In the curarized cat one hypoglossal nerve was tetanized for 15 minutes, the other meanwhile being left at rest. At the end of this period the curare block was gradually removed by a slow intravenous injection of physostigmine. The rate of recovery on the two sides was compared by alternate stimulation of the nerves with single shocks. Conduction from nerve to muscle was always restored first on the side of the tetanized nerve.

The procedure followed in these early experiments will first be given in more detail. The cat was anesthetized with sodium barbital (0.35 gram per kilo) or with nembutal (40 to 50 mgm. per kilo) given intraperitoneally. Shielded platinum electrodes, connected to a Harvard inductorium, were applied to each hypoglossal nerve, just peripheral to the point at which it crosses the carotid artery. The secondary coils were so adjusted that the break shocks were just above the strength required to cause a maximal contraction of the tongue muscle. With 3 volts in the primary circuit, the secondary coil was usually set at 11 cm. and at an angle of 50 to 70 degrees from the horizontal. Once adjusted, it was left in the same position throughout the subsequent procedure.

The cat was then curarized, by intravenous injection at intervals of small amounts of a 1 per cent solution of crude curare. This came from three different samples, of varying potency (Merck, and Burroughs, Wellcome & Co.) For complete loss of indirect excitability of the tongue muscles, the amount required varied from 2.5 to 7.0 cc., and an excess of 0.5 to 1.5 cc. was given. The nerves were tested alternately from time to time with single shocks, and contractions usually ceased almost simultaneously on the two sides. The jaws were kept open so that the tongue could be observed directly. As a rule the contractions were also recorded, by means of a single muscle lever, but this was not sufficiently sensitive to respond to the smallest visible twitch. Sometimes a feeble contraction

would persist longer on one side than on the other, and more curare was required for a complete bilateral block. Artificial respiration was started when the onset of paralysis rendered it necessary, and was maintained at a uniform rate thereafter.

When the block was complete on both sides, one nerve was tetanized, 100 interruptions per second, for several minutes. Ordinarily we selected the right nerve for tetanizing in one experiment and in the next the left, regardless of any difference in initial susceptibility to curare.

For the sake of brevity, we shall refer to the tetanized nerve and its muscle as nerve and muscle A, and to the opposite resting nerve and its muscle as B. As soon as the tetanizing was stopped, both nerves were tested immediately, each with a single break shock. The slow injection of physostigmine was then started, from a burette attached to the cannulated femoral vein. The solution used contained 1 mgm. of physostigmine sulphate and 0.5 mgm. of atropine sulphate in 2.5 cc. of 0.9 per cent NaCl. The rate of injection was varied in different experiments, but averaged about 0.5 cc. per minute. In the individual experiment the rate was not entirely constant, because of the falling level in the burette. During the injection the nerves were tested alternately, at intervals of 10 to 20 seconds.

The above procedure has been followed in 18 experiments. The duration of the tetanizing stimulus was varied from 5 to 15 minutes. The results were all alike. The time required for recovery, on both sides, varies according to the depth of curarization, and (especially for B) according to the rate at which physostigmine is injected; but it is always much shorter for A than for B. A typical graphic record is shown in figure 1. We can make no statements regarding the comparative effects of tetanizing with frequencies other than 100 per second. At this frequency, however, stimulation for 5 minutes seems to be at least as effective as for the longer periods. In 8 instances muscle A responded to the first test stimulus applied to its nerve, immediately after tetanizing ceased. This was before any physostigmine had entered the vein.

It may appear singular that muscle A, inactive during tetanization of its nerve, should contract in response to a single shock of the same intensity applied immediately afterward. This is apparently a typical Wedensky "inhibition." It differs from the classical demonstration in that there is no initial contraction at the beginning of the series of tetanizing stimuli, the preparation being at that time incapable of responding either to single shocks or to a succession of them.

Temporary removal of the curare block can be accomplished by much shorter periods of tetanizing, and the use of physostigmine is not necessary. The cat is curarized very gradually, until a single shock applied to either hypoglossal nerve causes no visible contraction of the tongue muscle. One

nerve is then tetanized for 5 to 30 seconds. Depending on the degree of curarization, this may elicit *a*, no visible effect; *b*, an initial twitch only; or *c*, a weak tetanus, highest at first and rapidly sinking. (Such a response to a series of properly timed indirect stimuli, each maximal for the nerve, at a stage when one fails to cause a muscular contraction, is the well-known "addition latente" of Bremer.) But if, following the tetanization, single break shocks are applied at intervals of 5 seconds or so, contractions usually appear. With some irregularities, these tend to become stronger for a time and then to fade out in a descending staircase, the complete curare block being finally reëstablished. Two typical records are shown in figure 2. We have also carried out this type of experiment, with similar results, on the sciatic nerve during occlusion of the abdominal aorta.

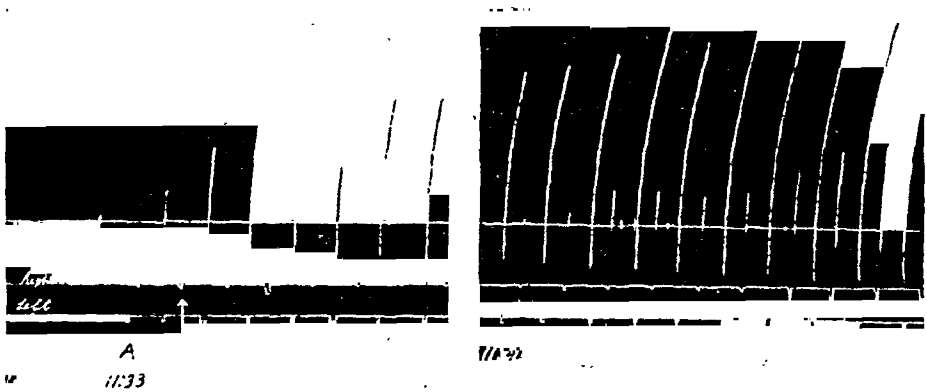

Fig. 1. Curarized cat. Above, contractions of tongue. Upper signal, stimulation of right, and lower signal, stimulation of left hypoglossal. At *A*, the end of a 6-minute period of tetanizing the left nerve. At arrow, beginning injection of physostigmine. Note response to the first single shock applied to the left nerve, and first response to right 8 minutes later. Interval between stimuli (for each nerve) 30 seconds. There is a gap of 4 minutes between the sections of the figure.

This effect has doubtless been observed before. Much attention has been given to other phenomena seen in the intermediate degrees of curarization, particularly summation effects and the several stages of the Wedensky inhibition. But I have found no mention of this temporary restoration of excitability of the muscle toward single indirect stimuli. With resting intervals of 15 to 20 minutes, it can be demonstrated repeatedly on the same nerve-muscle, at least under the conditions described.

It is evident that tetanization of the hypoglossal nerve accelerates in some way removal of the curare block. The effect could be explained by assuming that the impulse conducted over a previously active nerve differs in some way from an impulse conducted over a nerve previously at rest, and that the former is better able to pass through the resistance set up

by curare. The available evidence seems to conform best to this explanation, though there are obviously others to be considered.

The first is that stimulation of the nerve may lead to local vascular changes, favoring either the elimination of curare from the muscle or the access of physostigmine to it. This hypothesis appears untenable, for the following reasons:

a. As explained above, physostigmine is not necessary for the recovery of the preparation.

b. The arterial supply may be interrupted in short experiments, as mentioned, without affecting the result.

c. In four curarized cats, with the blood rendered incoagulable by heparin, we have recorded, by the drop method, the outflow of blood from the lingual vein. Tetanizing the hypoglossal, with stimuli of the same

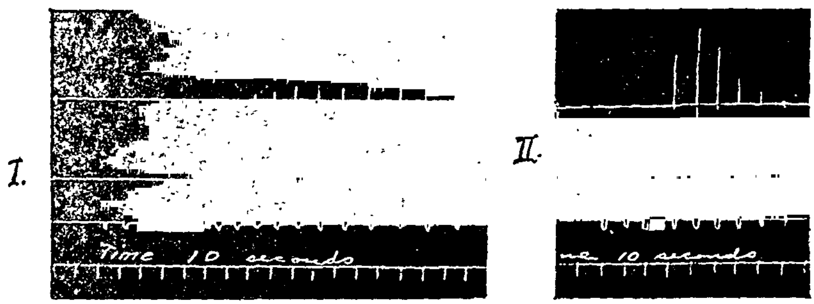

Fig. 2. Curarized cats. Order of record as in figure 1, with addition of time tracing (10 second intervals) below. Showing in each record *a*, initial absence of response to single break shocks; *b*, a period of tetanizing, likewise without visible effect; and *c*, the temporary return of indirect excitability, with final relapse into complete block. In the animal used for II, the left superior cervical ganglion had been excised 18 days previously.

range of intensity used in the experiments described above, caused no change in the total volume flow through the tongue.

d. We have excluded, by control experiments described below, the sympathetic fibers, which constitute the only known vasomotor pathway in the hypoglossal. Van der Sprenkel (1924; also Langworthy, 1924) suggests that the fibers from Roller's nucleus are vasodilators, but I have not found in the literature any experimental support for this view. The control experiments mentioned in *c* seem to prove the absence of any significant vasodilatation here. Reflex effects have been excluded by sectioning or tightly ligating the nerves (6 animals) central to the electrodes.

e. In a lightly curarized animal, stimulation of the nerve might cause contractions too minute (especially if the muscle fibers involved lay deep within the substance of the tongue) to be visible. Such contractions might influence the circulation locally, either mechanically or through metabo-

lites. This effect could not be great, however. And the order of recovery was always the same regardless of the depth of curarization.

2. A second hypothesis involves the sympathetics, though not as vasomotor nerves. Orbeli and Ginetzinsky (cited by Lapicque, 1930) report that stimulation of the abdominal sympathetic trunk, in curarized frogs, may temporarily restore indirect excitability to the muscles of the hind limb. The experiments have been repeated and confirmed by L. and M. Lapicque (1930), who find that sympathetic stimulation leads to a shortening of the chronaxie of skeletal muscle. In this connection may be recalled also the studies of Gruber (1914) and others on antagonism between curare and adrenalin, and the recent demonstration by Cannon and Bacq (1931) of an adrenalin-like substance liberated at the endings of stimulated sympathetic nerves.

The effect of hypoglossal stimulation in our experiments is strikingly like that of the sympathetic as found by Orbeli. We are convinced, however, that the sympathetic fibers contained in the hypoglossal have nothing to do with our results.

First, the absence of an effect on blood flow supports the belief that the stimuli used were too weak to excite the sympathetic fibers. Lapicque states that it was necessary to employ, on the frog's sympathetic, stimuli much above the strength found maximal for the somatic motor fibers of the sciatic.

Second, we have in four experiments left both hypoglossals at rest and tetanized the cervical sympathetic trunk on one side with strong shocks (sufficient to cause dilatation of the pupil and retraction of the nictitating membrane). Subsequently the hypoglossals were tested with single stimuli as usual during the injection of physostigmine. In all these instances recovery took place first on the side *opposite* to the tetanized sympathetic. In no case, however, was the difference in recovery rate so marked as it is following tetanization of one hypoglossal. In comparing these findings with those of Orbeli, it must be remembered that his experiments were made on the frog and that vascular influences were definitely excluded. Stimulation of the cat's cervical sympathetic undoubtedly caused vasoconstriction in the tongue.

Third, we have in three cats removed both superior cervical ganglia (in separate operations) and carried out the usual experimental procedure on the hypoglossals after time had been allowed for the postganglionic fibers of the sympathetic to degenerate. The results were similar to those obtained with normal cats.

The recovery of conduction between the tetanized nerve and its muscle evidently does not depend on any influence of the sympathetic, nor upon vascular factors. It seems probable that the conduction mechanism is affected in a more direct way.

Bremer (1929; also Bremer and Homès, 1931) finds that in a certain stage of curarization two nerve impulses, if properly timed, may cause a muscle contraction where one fails to do so. This he compares to a summation of direct stimuli. The effective period is limited to a small fraction of a second. There is no evidence that the phenomenon is modified by the previous rest or activity of the nerve, except as it involves the refractory period left by the first of the two impulses. The effect with which we are dealing here is not so brief, but may last at least for several minutes. It may, however, be fundamentally related to the "addition latente."

According to Lapicque's theory of isochronism, there are four ways in which a block of the curare type can be accomplished. Agents are known which fit three of these possibilities. The fourth is still only theoretical, as there is no agent known which will lengthen the chronaxie of nerve without similarly affecting that of muscle (Lapicque, 1926, p. 276). This work was originally planned with the idea in mind that fatigue might lengthen the chronaxie of nerve, as it does that of muscle, and therefore that tetanization of the nerve, in a curarized preparation, might aid in restoring conduction between them. Previous activity in nerve does prolong the action potential (Amberson, 1931) in a way which might conceivably render it more effective for exciting a curarized muscle. But it has been reported in another paper (Boyd and Gerard, 1930) that tetanization of frog nerve leads only to an elevation of the rheobase, with little or no change in chronaxie. And the recent work of Rushton (1931) throws doubt on the isochronism theory.

If the restored conduction depends on "fatigue" changes in the nerve, we might expect the preparation to lapse into a state of block again if it is simply allowed to rest. Providing physostigmine has not been administered, it actually does so, as illustrated in figure 2. Moreover, the duration of the temporary recovery varies in general according to the duration of the preceding tetanization (for periods up to one minute, at least). It seems difficult to explain this behavior on any basis other than that suggested. The fatigue changes in metabolism and heat production appear to have somewhat similar time relations (Gerard, 1927).

If this type of facilitation is a property of curarized preparations in general, it would seem strange that such an effect could have escaped notice in all the work which has been done with frogs. We originally chose to work with cats, because we had no idea that tetanization of the nerve would alone remove the curare block. We expected to require the aid of some antagonistic drug, and knew of none which will remove curare paralysis in the frog as rapidly and completely as physostigmine does in the cat. In a few experiments with frogs we have found no recovery of conduction after tetanizing the motor nerves. This negative finding may depend on the frequency and duration of the stimuli employed, or on a fundamental difference of behavior between frog and mammalian tissues.

SUMMARY

1. Tetanization of the hypoglossal nerve in curarized cats, for periods of 5 to 15 minutes, facilitates restoration of conduction between the nerve and the tongue muscle. This was demonstrated by comparison with the opposite resting nerve during slow intravenous injection of physostigmine.

2. In lightly curarized preparations simple tetanization of the nerve may restore indirect excitation of the muscle, without the use of physostigmine.

3. This effect is not due to vascular changes in the tongue, nor to any influence of sympathetic fibers in the hypoglossal nerve.

4. The suggestion is offered that previous activity in a nerve may so modify the propagated disturbance as to make it more effective in passing a curare block.

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AUTONOMIC DISCHARGES ELICITED BY PHYSIOLOGICAL STIMULI IN MID-BRAIN PREPARATIONS

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Karplus and Kriedl (1), Beattie (2), Hasama (3) and others have demonstrated by localized faradization the presence of nerve cells in the region of the hypothalamus having synaptic connections with the autonomic outflow. Beattie (2) recently, by degeneration studies, described fibres passing from the caudal hypothalamus to the thoracic cord. What functional rôle is played by these cells in the intact animal? The work of Isenschmid and Schnitzler (4) indicates clearly that integrated heat regulation is maintained in the rabbit with only the hypothalamus, mid-brain, pons, medulla and cord remaining intact (hypothalamic animal). The fact that removal of the hypothalamus (mid-brain preparation) eliminated heat regulation in their animals strongly suggests that integrated heat regulation is dependent on the hypothalamus. Bard (5) showed later that normal sympathetic tone of the eye structures, and the mechanism of exhibiting signs of intense rage are still intact in the hypothalamic cat (acute). He concluded that these functions are largely dependent on the hypothalamus, pointing out and stressing, however, the fact that sympathetic discharges are still obtainable in mid-brain preparations (quoted Cannon and Rapport, and Bazett and Penfield).

It at once becomes apparent that in order to understand more fully the function of the hypothalamus in its relation to the autonomic outflow, it is necessary to gain a clearer insight into the character of the autonomic mechanisms remaining intact in the mid-brain preparation. In order to simulate the intact animal as nearly as possible, chronic preparations have been resorted to and the responses (efferent discharges) studied are those that can be elicited by physiological stimuli (activity of animal, pulling and rubbing the skin, change in environmental temperature, etc.) rather than by faradization of an exposed nerve trunk.

Procedure. *Method of decerebration.* The superior colliculi were exposed dorsally under full aseptic precautions by retracting one or both of the occipital poles lateralad and cephalad. The lesion was made by means of a blunt instrument passed ventrally into the brain-stem at what was judged to be the desired level, care being taken that the instrument

passed under the vein of Galen. In all cases the tissue cephalad to the section was allowed to *remain in place* with intact blood supply (figs. 1 and 2). It was seldom that any appreciable bleeding resulted from the placing of the lesion even though the *carotid arteries were never tied, nor were the vertebrals ever pressed.* The main source of bleeding was met in going through the skull and in retracting the occipital poles from the collicular bodies. Bleeding was never severe and was readily controlled with wax and dry cotton pledgets. The first animals were prepared under amytal anesthesia, the rest being prepared under nembutal. Postoperative recovery is much more rapid and satisfactory with nembutal (40 mgm. per kilo.).

Postoperative care. The preparations were routinely given 50 cc. of saline subcutaneously directly following the operation and then placed in

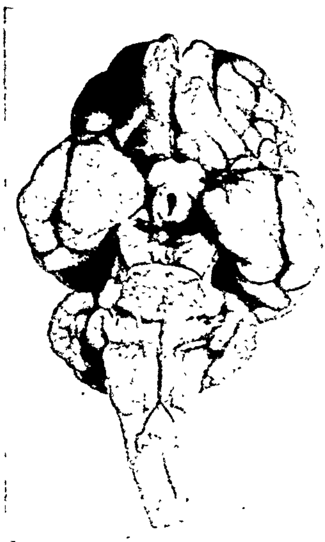

Fig. 1

Fig. 2

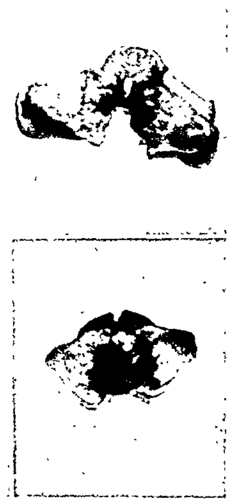

Fig. 3

an incubator. The following day, as well as on subsequent days, they were fed an egg and milk mixture by stomach tube.

It was found better to maintain the animal's temperature slightly below (at approximately 37°C.), rather than above normal. The margin of safety in over-heating is much less than in over-cooling. Death occurs readily as a result of over-heating. The rectal temperature mounts rapidly over a relatively short period of time with only a slight rise in environmental temperature. Preparations, on the other hand, have been maintained at from 32°C. to 37°C. for hours without any apparent injury; furthermore, the rectal temperature can be lowered for short intervals in the ice-box to 28 and 27°C. without any immediate danger.

The success of maintaining the animals in our experience was limited

by two factors: first, the amount of trauma produced (ultimate necrosis) in placing the lesion, and second, the onset of respiratory tract infection.

The animals that deteriorated as a result of excessive trauma from the lesion showed progressive increased respiration and atonic anal sphincters 12 to 24 hours before death. The symptoms of deterioration made their appearance in from 2 to 5 days after operation. The nearer the lesion was to the tuber cinereum, the more trauma the same instrument produced, the reason probably being due to the mechanics of the blood supply in this region.

Respiratory tract infection occurred as a rule any time during the first 3 to 4 days after operation. It has recently been successfully combated by using a large, unrounded stomach tube too large to enter the trachea, and by routine periodic stretching and massaging every 4 to 6 hours. This periodic treatment serves also to keep the intestinal movements active. A constipated preparation is sluggish and the responses obtained are *greatly lessened and may even be entirely negative.*

As soon as there were any suspicions of deterioration the animals were checked closely and terminated. The brain was removed, hardened in 10 per cent formalin, and blocked. Serial sections were made of the block of tissue caudal to the transection and stained with iron hematoxylin for the purpose of determining the level of the transection.

Excluding several preliminary experiments, twelve preparations have been studied, two of which survived 18 and 20 days respectively. Both of these animals were killed by accidental over-heating. The transections ranged in the different preparations from at just the cephalic level of the mid-brain to the caudal level of the mid-brain. In some cases medial necrosis extended well back in the cephalic pons.

Observations. *Sympathetic tone of the eye structures.* A comprehensive, historical review of the literature bearing on the functions of the cervical sympathetic has recently been given by Fulton (6). The normal tonic activity of the cervic sympathetic trunk is evident from the fact that when the nerve is cut on one side the following marked changes occur on that side ("Horner's syndrome"): 1, constriction of the pupils; 2, decrease of intraocular pressure; 3, sinking in of the eyeball (enophthalmus); 4, drooping of the eyelid; 5, narrowing of the fissure between lids; 6, covering of the eyeball by the nictitating membrane, and 7, dilatation of the blood vessels of the eye and face.

If the tonic action originates from a hypothalamic level, it was believed that a hemisection through the brain-stem anywhere caudal to the hypothalamus might result in a unilateral atonia. A hemisection at the cephalic level of the pons leaving the efferent constrictor fibres to the pupils intact as well as leaving the eye sensitized, did not produce a Horner's syndrome,

except that the pupils were unequal. The one on the side of the hemisection was smaller than the opposite one in an ordinarily lighted room, while in a dark room, the pupil on the side of the section became the larger. Both pupils, however, dilate maximally and equally when the eyes are atropinized; whereas, in an intact animal with one cervical sympathetic cut, dilatation of the enervated pupil after atropinization is much less than in the opposite eye.

The fact that Horner's syndrome is not produced by such a section does not indicate that sympathetic tone is independent of the hypothalamus, since half the brain-stem remains intact. Karplus and Kriedl (1) noted bilateral pupillary dilatation from unilateral stimulation of the hypothalamus. Hasama (3) recently stated that bilateral sweating is obtained from unilateral stimulation of the hypothalamus. Further, an animal maintains tonic bilateral integrated heat regulation with the brain-stem hemisected at any level through the upper medulla, pons and mid-brain (7), and even when the hypothalamus is completely removed on one side (8). These facts indicate that each unilateral hypothalamus has bilateral connections with the autonomic outflow from the spinal cord.

It then became of interest to see if normal sympathetic tone of the eyes remained intact when the hypothalamus was eliminated by a complete transection of the brain-stem. Advantage was taken of cutting the cervical sympathetic nerve on one side, thus allowing for a *completely atonic control with which to compare the innervated eye.*

The procedure of observing the animals carefully 24 to 72 hours following decerebration was found necessary because frequently unilateral edema, traumatic corneal irritation and other resultant operative factors produced an asymmetry in the eyes which simulated rather closely the picture of unilateral nerve section. After the decerebration was found to be satisfactory the cervical sympathetic nerve was cut on one side under local anesthesia.

Low transections. An animal with the section at the middle level of the mid-brain shows no spontaneous activity and when at rest the nictitating membranes are over the eyes, the lids are closed or nearly so, and insofar as can be judged, the balls are sunken and soft. Usually the section destroys the constrictor nuclei of the pupils. In this case, the pupils remain from 8 to 10 mm. in diameter. When the section passes caudal to the constrictor nuclei the pupils remain slits.

Manipulation of such an animal elicits tonic sympathetic activity judged by the opening of the lids, dilatation of the pupil, withdrawal of the nictitating membrane, the intraocular tension becomes greater and the balls protrude more in the innervated eye, while these structures in the enervated eye remain stationary.

Active response in the innervated eye as against no response in the enervated eye demonstrates conclusively the passing of efferent discharges from the cord to the eye by way of the cervical sympathetic.

The amount of manipulation necessary to elicit discharges to the eyes varies somewhat with different preparations, and further with the same preparation over successive days. Cat 225 is typical for a low transection. When completely at rest both eyes were atonic as in normal sleep; merely placing the hand on the head to examine intraocular tension resulted in active raising of the head with a simultaneous appearance of some tone in the intact eye. Picking up the animal in the hands at once resulted in complete sympathetic tone; this was frequently associated with mild sissing or snarling.

These preparations executed definite clear-cut standing, stepping and righting reactions, even though the rubro-spinal systems were completely degenerated, which will be discussed in detail in another report. Typical extensor rigidity was never observed.

The following note from the protocol of cat 225 illustrates the reaction of the animal:

7th day postoperative. At rest, without handling, there was a definite slight difference in the lids and nictitating membranes, the left lids being narrower and the left nictitating membrane being higher over the eyeball. Both pupils were 10 mm., and the left ear vessels were moderately dilated, the right ones being streaks +. The cat began coughing spontaneously. This was associated with a complete active standing of the animal (it was already righted with the abdomen against the floor); right lids opened $\frac{3}{4}$, the nictitating membrane withdrew nearly out of sight, while the left lids and nictitating membrane remained stationary. Then, following rage, the right lids opened completely and the nictitating membrane was completely withdrawn, while the left lid remained narrower and the nictitating membrane remained over the eye.

20th day. Cat was accidently killed by overheating.

Autopsy. The head was well healed. The brain was in excellent condition, there being no softening in the occipital poles. The fresh brain showed no evidence of a lesion except ventrally the level of the section could be made out by careful inspection. The pia with its enmeshed vessels was intact.

Microscopic findings. Necrosis extended caudally along the midline on the left, involving the exiting root of the oculomotor nerve. The cephalic portion of the oculomotor on the right was encroached upon. At the maximum width of the lesion there remained intact on both sides the extreme caudal tip of the medial geniculate body and a small lateral portion of the cerebrospinal tracts. This is readily seen from figure 3.

High transection. The animals with transection through the extreme caudal end of the hypothalamus (caudal tip of mammillary bodies), which *eliminates the hypothalamus entirely* and leaves practically the whole of the mid-brain intact, at times exhibits a characteristic latency before sympathetic tone appears in the eye. At rest, the animals show complete

atonia of the eyes with the pupils as slits. Unlike the cats with lower sections, these animals, even when in good physiological condition, could be picked up and carried without waking, *e.g.*, without the appearance of tone in the innervated eye. However, when stimulated sufficiently to elicit activity, both pupils dilated readily, the innervated one always being the larger of the two, the lids opened, the innervated nictitating membrane withdrew readily and completely. It seemed as though these cats had some sort of an inhibitory mechanism that protected the animal from being aroused by manipulation. However at times they *exhibited the same readiness of response* as did the cats with sections further caudad.

Spontaneous tone, insofar as it occurred without peripheral stimulation, appeared as a result of the spontaneous activity of the animal. This activity was characterized by successful movements of progression with the body near the floor and the shoulder blades projecting slightly above the dorsum of the back. Movements of progression also in some cases followed rage-eliciting stimulation.

The following note is from the protocol of cat 226:

2nd day. 8:30 a.m. When at rest the pupils were approximate slits; lids $\frac{3}{4}-\frac{1}{3}$ open; nictitating membranes $\frac{3}{4}$ over the eyes. After being aroused the left pupil was 6 mm., right 7 mm.; lids $\frac{3}{4}+$ open; nictitating membranes $\frac{1}{6}$ over the eyes.

12:25 p.m. Pupils dilated when thermometer was inserted into the rectum.

2:00 p.m. Cat was placed on floor after which it made an active attempt to walk, taking a few steps with the abdomen near the floor. It soon rested and *exhibited all the typical signs of sleep*; the pupils were slits, and one could pick up its head and move it about without arousing the animal or causing the pupils to change.

6:30 p.m. Cat still sleeping (pupils slits) and remained so when handled, until a growl was elicited, at which time the pupils dilated.

6th day. 11:00 a.m. (Just previous to the cutting of the cervical sympathetic nerve on the right.) The cat walked about on floor readily. As usual, it showed spontaneous activity in walking and walked upright in a normal position.

7th day. 9:00 a.m. Left pupil 10 mm., right 9 mm. Left nictitating membrane completely withdrawn, right $\frac{1}{3}$ over the eye. Left lids $\frac{3}{4}-$ open, right $\frac{1}{3}$ open. The cat died in a convulsion during examination. At death the left pupil was 13 mm., right 10 mm. Left nictitating membrane completely withdrawn, right $\frac{1}{3}$ over the eye.

Autopsy. Ventrally, the pia and the blood vessels were intact; however, it was readily seen that the whole of the hypothalamus was necrotic and that the section had passed just caudal to the mammillary bodies.

Microscopic findings. The lower level of necrosis in the stem was back of the caudal level of the posterior commissure dorsally and at the cephalic level of the exiting oculomotor nerves ventrally. Intact tissue reached slightly further cephalad on the right than on the left side.

Outward rage signs. Rage is readily elicited in animals with the section through the middle of the mid-brain, where constipation is ruled out. It is evidenced by sissing, growling, wagging of the tail, pawing movements, and erection of the hair and *simulates typical rage* (discharge of sympathetic as a whole) except the purposeful somatic element of "escape" and "de-

fense" is absent. Rage signs may appear simultaneously with the appearance of sympathetic tone in the eye as a result of a normally inadequate rage stimulus or may not appear until more vigorous stimulation is given, such as the abrupt pulling of the skin over the dorsum of the back.

With the section passing just at the cephalic level of the mid-brain, rage was, as a rule, more difficult to elicit than in a preparation with the section passing a millimeter or two further caudad. The animals with such sections correspond closely in their reactions with Bard's animals 97 and 50.

The preparations with sections more caudad simulated the description given by Bard of his animals where the sections were placed just caudal to the optic chiasm; the essential differing factor being that in our preparations strictly spontaneous rage has never been observed, and when rage was elicited it persisted only as long as stimulation continued. The stimulation threshold varied to some extent in different preparations and in the same preparation at different times. Their great reactivity (low threshold) is demonstrated in cat 225 when growling was elicited as a result of its own sneezing.

Starling states in his textbook that vocalization occurs in a mid-brain animal. He makes no mention of the nature of the response. Bazett and Penfield (9) report observing rage responses in their chronic mid-brain animals which were prepared by removing the tissue ahead of the section.

The secretion of adrenalin. The fact that some chemical is liberated into the circulating blood at the time that outward rage signs appear (very likely adrenalin) is evident from the fact that when rage is elicited continually over a period of 30 seconds to 2 minutes, the sympathetic effectors of the enervated eye respond maximally and equally to the innervated structures, and the ear vessels constrict in the enervated ear. The chemical substance responsible simulates adrenalin further in that hyperglycemia, a rise in blood fat, a profuse secretion of saliva result and glycosuria follows. It should be pointed out that the adrenalin-like substance is not liberated in sufficient quantity to produce these results until a considerable latent period following the onset of sympathetic tone in the eye and following the first appearance of outward signs of rage.

Cannon and Rapport (10) obtained a reflex secretion of adrenalin in acute preparations by stimulating the central stumps of cut-nerve trunks, with sections corresponding to ours dorsally. They made no mention of the level of their sections ventrally. Brooks (11) recently reported obtaining reflex hyperglycemia in acute medullary preparations. The following notes are from the protocols of cats 200, 221 and 225:

Cat 200. 17th day. 10:10 a.m. Blood sample taken. Sugar 111 mgm. per cent; fat 698 mgm. per cent.

10:12 a.m. Animal was irritated by massive pulling of skin along the back. Ani-

mal responded very vigorously by growling, sissing, spitting, and erection of hair over the back and tail.

10:13 a.m. Left pupil 11 mm.; right 10 mm.

10:18 a.m. Left pupil 12 mm.; right 11 mm.

10:27 a.m. Left pupil 12 mm.; right pupil also 12 mm. Eliciting of rage was stopped at this time.

12:29 p.m. Second blood sample was taken. Sugar 108 mgm. per cent; fat 934 mgm. per cent.

5:00 p.m. Urine contained 4.7 mgm. sugar per cc. urine.

20th day. Autopsy. The cat was killed by overheating. There was no evidence of a lesion ventrally until, after hardening, the intact pia with its embedded vessels was removed. The brain-stem was completely transected except for a lateral strip consisting of the medial geniculate body on the right.

Microscopic findings. Fibre tract and cell staining of serial sections through the block of tissue caudal to the transection exhibits necrotic tissue along the midline well back of the cephalic level of the pons. As the sections extend further cephalad the necrotic area widens out laterally to include the whole of the left cerebrospinal tract and the medial $\frac{2}{3}$+ of the right cerebrospinal tract at the cephalic level of the pons. Sections from the upper medulla, lower medulla, lower cervical and middle thoracic cord stained by osmic acid revealed *consistent bilateral degeneration of the cerebrospinal, rubrospinal and tectospinal systems.*

Cat 221. 3rd day. 8:00 a.m. Abrupt pulling of the skin over the back and ribs (first attempt to elicit rage) caused the animal to growl angrily. When given milk by stomach tube the animal fought the tube with struggling movements with the claws and biting movements with the jaws.

3:30 p.m. Left cervical sympathetic cut under aseptic conditions. The exposure of the nerve elicited struggling and marked growling.

4th day. The animal growls and sisses readily when skin is pulled slightly over the dorsum of the back.

5th day. 3:00 p.m. Blood sample taken from jugular vein. Sugar 241 mgm. per cent; fat 849 mgm. per cent. Urine sample collected at this time by pressing from the bladder, sugar 2.27 mgm. per cc.

3:12 p.m. Eliciting rage was begun by pulling skin abruptly over the dorsum of the back.

3:17 p.m. Left pupil 10 mm.; right 8.5 mm.

3:26 p.m. Left pupil 11 mm.; right pupil 9.5 mm.

3:32 p.m. Left pupil 10 mm.; right 8 mm. Left ear vessels moderately dilated, right ear vessels were streaks. Rage-eliciting was stopped and a blood sample taken from the jugular vein. Sugar 287 mgm. per cent; fat 1182 mgm. per cent.

Autopsy. The animal was accidentally drowned on the 6th day while being fed. Blood stains were still evident over the occipital poles, the bony tentorium and the superior colliculi. None of the ventral vessels were disturbed, nor was the pia torn.

Microscopic findings. Iron hematoxylin staining of serial sections through the block of tissue caudal to the section revealed necrosis medially back of the cephalic level of the pons. At the upper limits of the pons the necrosis extended 2 mm. laterally from the midline on each side. The necrosis quickly widened to involve the whole stem. A few lateral cells in the caudal tip of the red nucleus survived on one side.

Cat 225. 7th day. Sissing and growling were more easily elicited this morning than previously, or than observed in any other preparation. Merely picking up the animal elicited marked rage signs and at *one time the animal sissed of its own accord,*

but it seemed to occur as a result of the stimulation induced by a sneeze. The cat cannot be manipulated in the least without rage resulting.

17th day. 3:00 p.m. Cat at complete rest: Both pupils 11 mm. (both atropinized). Nictitating membranes over the eyes equally. Lids approximately equal. Left ear vessels slightly dilated, right ear vessels streaks.

3:02 p.m. Mild stimulation: Left pupil 11 mm.; right pupil 12 mm. Left nictitating membrane ⅓ over eye, right nictitating membrane was withdrawn.

3:07 p.m. Marked stimulation: Both pupils 13 mm. Eyeballs seemed equally tense and showed normal pressure. Marginal ear vessels streaks in both ears. Saliva dripped from the mouth.

3:12 p.m. Both pupils 13 mm. +. Left ear vessels streaks +, right ear vessels streaks (adrenalin?). The stimulation was stopped at this time.

3:15 p.m. Both pupils 12 mm.

3:18 p.m. Both pupils 11 mm. Nictitating membranes ⅔ over eyes. Left ear vessels slightly dilated, right streaks.

Microscopic findings. See previous description.

Vasomotor tone as observed from the ear vessels. As has been mentioned previously, vasomotor tone is present in the ear vessels even when the animal is completely inactive; however, in a few instances, it was definitely noted that the ear vessels constricted slightly simultaneously with the appearance of sympathetic tone in the eye structures. This indicates clearly that vasomotor tone is independent of afferents aroused by the activity of the animal (probably maintained by afferents from the blood vessels themselves); yet, on the other hand, it is definitely influenced by such afferents (activity of animal).

Our sections were several millimeters above the middle of the pons which is the cephalic level of the central vasotonic mechanism, as was first reported by Dittmar (12).

Another interesting and clear difference between an intact animal and a mid-brain preparation was noted in their reactivity to environmental temperature. In a normal cat when its rectal temperature is raised by overheating in an incubator, the ear vessels dilate maximally and remain so dilated, when the animal is removed from the incubator, until the rectal temperature has returned to normal. In a mid-brain preparation overheating dilates the ear vessels as in an intact animal; however, the ear vessels constrict rapidly on removal from the incubator, even into an ordinarily warm laboratory, whereas the rectal temperature remains elevated. This difference in reactivity can be especially demonstrated if the animals are removed directly from the incubator to a relatively cool room. The following notation from the protocol of cat 200 demonstrates the reaction:

10th day. 9:47 a.m. Cat placed in hot-box at 49°C.

11:50 a.m. Respiration 21. Temperature 42.8°C. Both ear vessels markedly dilated. The smaller right angle vessels showed up well in both ears.

11:58 a.m. Respiration 40. Temperature 43.8°C. Ear vessels as previously

described. When the tongue was pulled forward by a pair of forceps, a fit of rapid breathing occurred. I was unable to make a count. At this time the cat was removed to the animal room with a temperature of approximately 25°C.

12 noon. Temperature 44°C. Ear vessels were streaks +. The vessels were observed to constrict down characteristically and rapidly.

Discussion and conclusion. The foregoing results demonstrate that efferent autonomic discharges, producing activity in the structures of the eye innervated by the sympathetic, can be elicited by physiological stimuli in animals with the brain-stem sectioned through the middle level of the mid-brain. The discharges are the same as those occurring in the intact animal insofar as can be judged by the effectors stimulated. We are dealing with discharges passing over the cervical sympathetic nerve, since cutting one of the nerves in the neck results in the typical Horner's asymmetry.

It is to be noted that sympathetic tone in the eye structures was only present when the animals were aroused either by stimulation or spontaneously. Spontaneous activity occurred only in animals with high sections. A further note of importance is the fact that in preparations in good physiological condition the *stimulus necessary to induce tone was slight.* Since sympathetic activity in the eye was readily induced as soon as the animal recovered from the anesthesia, it is not believed that the mechanism suffered from any appreciable depression as a result of the transection.

The only clear-cut conclusion that can be drawn from these observations is that *the sympathetic tone of the eyes induced by physiological stimuli is not dependent on the brain-stem ahead of the middle level of the mid-brain.* This does not necessarily add to nor detract from the evidence upon which the conception is built that the autonomic cells in the hypothalamic region mediate an integrative action over the tonic sympathetic control of the eye structures.

A *typical rage response* can be obtained in a preparation with only the cord, medulla, pons and small caudo-lateral portions (essentially a pontile animal) of the mid-brain remaining intact. The end response differs from that obtained in a normal animal only in that the somatic effectors are not coördinated into an "escape" or "defensive" pattern. The animal responds by sissing, spitting, growling, tail-lashing, aimless pawing or clawing movements, and marked sympathetic activity as evidenced by the elevation of hair, dilatation of pupils, protrusion of eye-balls, and with an increased output of adrenalin. Not only has the response been induced by stimulation such as is ordinarily rage-producing, but also by slight disturbances such as gently picking up the preparation and even the animal's own sneezing. The placing of the section seems not to depress the reactions. The absence of the "protective" or "defensive" pattern is probably best explained as due to the absence of central connections between local-

izing afferents with somatic outflows. For instance, such an animal lacks sight, hearing, smell, and the ability to localize disturbances in the periphery, *i.e.*, pertaining to the body surface ("local sign").

Here again the only conclusion that can be drawn is that *the discharging of a typical rage response is not dependent on the brain-stem cephalad to the middle level of the mid-brain.* This fact, however, does not eliminate the possibility of an additional central mechanism located cephalad (hypothalamus) playing a rôle in the discharging of efferent impulses during rage in an intact animal.

The observations reported on the vasomotor responses resulting from a change in environmental temperature indicate that *in the mid-brain preparation the afferents from receptors in the skin dominate any possible direct action of the temperature of the blood* acting either on efferent cell bodies in the stem caudal to the section, or on internal peripheral receptors. This is evidenced by the ready constriction of the ear vessels when the skin is subjected to a cooler environment, even though the temperature of the blood (rectal) remains markedly above normal (as high as 44°C. in one instance).

SUMMARY

1. Chronic mid-brain and pontile preparations have been maintained for as long as 20 days. The cerebral hemispheres and brain-stem ahead of the section remained in their normal position with an intact blood supply.

2. In these preparations normal sympathetic tone in the eyes and typical signs of rage were readily induced by physiological stimuli.

3. In the mid-brain animal the environmental temperature determines the state of constriction of the cutaneous vessels rather than the temperature of the blood (rectal temperature).

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AN INQUIRY INTO THE BASIS OF THE ACETYLENE METHOD OF DETERMINING THE CARDIAC OUTPUT

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Various respiratory procedures have been proposed for estimating the cardiac output. Of these the method of Marshall and Grollman (1) has perhaps received the most careful and critical substantiation.

As far as it need concern the present discussion, the method in its more recent development (2) involves the breathing into the lungs of a mixture of acetylene and air enriched slightly with oxygen. The mixture is re-breathed in and out of a rubber bag for at least 15 seconds, and a sample taken (at expiration?). A second similar sample is taken about eight seconds later. From the acetylene, oxygen and CO_2 content of these two samples and the resting O_2 consumption, the resting cardiac output is calculated by a formula which presupposes among other things, that the re-breathing procedures are not extended beyond such a time as may permit re-circulation of acetylene-containing blood to the alveoli.

In determining the cardiac output of man in the Louisville laboratories, by the injection method (3), one of us with Moore and Kinsman has come tentatively at least to the conclusion that the cardiac output is much more variable than it would seem according to Grollman's figures. Once in a while our output figures are low, as are those of Grollman, but in such experiments the total circulation time is long—18 or 20 seconds. In a great majority of cases, however, the total circulation time is shorter (10.0, 10.5, 12.0, 12.0, 12.0, 13.0, 14.0, 14.0, 15.0, 15.0, 15.0, 16.0, 17.0, 18.0, 18.0, 18.0, 18.0—av. 14.7 seconds) and the output greater than would be congruent with the results of Grollman. Total circulation times longer than the above list were usually associated with congestive heart disease, while total circulation times shorter than these were usually associated with severe anemia or hyperthyroidism (cf. Blumgart, 4). The fact that Grollman used practised subjects who were probably not anxious about the procedures, and the further fact that emotional excitement probably increases the cardiac output (14) makes the results from the two laboratories not quite as incongruent as they might appear at first glance. It may be that occasional subjects who are not worried will show a sufficiently

long circulation time to make possible the procedure of Marshall and Grollman. Such a situation is rendered improbable by the above list of circulation times, taken from patients who were not all of them unduly excited, and by the further fact that these figures are of the same order as (or somewhat less than) those of the arm to arm circulation time (Blumgart, 4) (av. 18 seconds). This condition arises from the fact that the arm to heart time is of the same order (6.6 seconds) as, or somewhat greater than, the circulation time through the shorter systemic paths. The fact that these shorter systemic paths carry very appreciable quantities of blood is evidenced by the measurements of Starr and Collins (5) who show that in 15 seconds 44 per cent of the cardiac output of the dog has passed through the systemic path and back to the heart. Comparison of the figures of Stewart (6) and Blumgart (4) who give 6.3 seconds and 6.5 seconds respectively as the average time of the pulmonary circulation in dogs and in man, indicates that these figures can be transferred almost bodily from dog to man.

Since the procedure of Marshall and Grollman involves vigorous breathing, and since this increases the circulation rate by 30 per cent (1) more or less, one would expect a similar decrease in the circulation time, and could not be justifiably surprised if, under the conditions of the experiment, blood began to return to the lungs in considerably less than 15 seconds.

Now if the blood which returns to the lungs after the first circulation and before the *first* acetylene sample brings back acetylene, it will have the effect of *decreasing* the cardiac output figure by *hindering* the diffusion of acetylene into the lung blood as well perhaps as by *raising* the relative value of the second acetylene sample and lowering the acetylene difference figure. We came to the conclusion, on the basis of experiments which are to be detailed below, that the first blood to return brought acetylene back with it. Before we could properly evaluate the significance of these experiments we had them confirmed, in part, for man at the hands of Grollman himself. Baumann and Grollman (7) showed that when a sample of right ventricular blood was taken between 13 and 20 seconds after acetylene breathing had begun, it was found to contain "five per cent acetylene." Data concerning the strength of the acetylene breathed, the type of breathing and the actual basis on which the five per cent figure rests, are unfortunately left for the reader to supply. From this single experiment it is implied that one should grant that merely insignificant amounts of acetylene, even under normal conditions, return to the lungs before 20 to 25 seconds. If the return of "five per cent" could be expected to work a change of five per cent or less upon the calculated output by merely interfering with the uptake of acetylene by the lung blood, the matter would be of little importance. One would merely say the results

might be five per cent or so too low and accept them as such. Unfortunately, however, the situation is more complex than this; the second sample might be affected more than the first, hence the difference between them would be reduced not merely by the general reduction of diffusion inwards but also by the specific effect of the returning acetylene upon the second sample. Since the difference figure is directly proportional—other things being equal—to the calculated flow, and since the figure itself is of the order of two or three per cent acetylene, it is impossible to conclude that a return of five per cent acetylene would affect this difference figure to a negligible extent.

Thus one is led to conclude that if the output is high and the total circulation time low, the method of Marshall and Grollman will give results that are too low, though it may be adequate in case the output is low and the total circulation time high.

EXPERIMENTAL. In order to throw additional light upon the time necessary for the diffusion of acetylene into the blood and again out into the lung air after it had been carried through the systemic circulation, it was decided to ventilate one lung with pure acetylene and the other lung with oxygen simultaneously, taking samples from the oxygen lung at various intervals and testing them for acetylene.

Sodium amytal was used as the anesthetic, since the use of a non-volatile substance was imperative. A tracheotomy was done and a glass tube inserted and fixed with ligatures. Artificial respiration was instituted when needed. The left bronchus was then exposed by resection of the fifth, sixth, seventh, and eighth ribs. A clamp was placed as near as possible to the origin of the left bronchus, closing off the left lung entirely from the right, and allowing the animal to be ventilated through the right lung only. The left bronchus was then cut distally to the clamp and intubated, producing air-tight connections. The circulations to the right and left lungs were not disturbed, and all hemorrhage was controlled. In this way, the air passages to the two lungs were separated from each other entirely, but their circulations left intact.

A bag filled with acetylene was then connected to the left bronchus. A bag of oxygen was connected to the tracheotomy tube by means of a thick walled rubber tube, into which 18 gauge needles, attached to evacuated gas samplers, were inserted. Simultaneously, every four seconds, both lungs were expanded by their separate gases, samples being taken at definite intervals from the tube leading to the right lung. The samples were taken at the end of expiration (complete collapse of lungs) and therefore were alveolar samples. They were analyzed for carbon dioxide and acetylene by the method of Treadwell and Tauber (8), as advocated by Grollman.

The data thus obtained from a series of four experiments were plotted

in figure 1, in which the ordinates represent the percentages of acetylene in the samples from the right (oxygenated) lung, and the abscissae the length of time for which the left lung had been ventilated with acetylene. Based upon preliminary experiments, the error of the analyses from all sources is probably not greater than ±0.025 per cent of acetylene, and the odds that the results from eight seconds on are significantly greater than zero are in all cases better than ten thousand to one. By the time the samples were taken the dogs were moribund and one could hardly expect as much acetylene to come off as did. Fortunately shock does not markedly increase the circulation time (unpublished data) though it diminishes the cardiac output and renders the animal unable to increase cardiac output under the severe anoxemia which probably obtained under the conditions of the experiment.

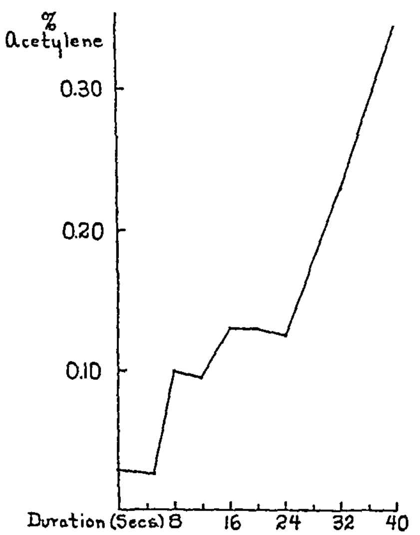

Fig. 1. Return of acetylene to one lung after being carried by the circulation from the other lung through the systemic pathways. Ordinates: concentration of acetylene; abscissae: time after the beginning of acetylene ventilation.

From the chart it can be seen that after re-breathing for eight seconds, a definite amount of acetylene is detectable in the oxygenated lung, indicating re-circulation of acetylene in this time. These results show a circulation time of the same order as the injection method under similar conditions (unpublished data) and definitely disprove the possible supposition that the first load of acetylene is all deposited in the tissues.

Since the stump of the left bronchus was closed off completely by the clamp, any acetylene found in alveolar samples from the right lung must have reached the lung through the circulation. If acetylene returns to the lungs in dogs in as little time as eight seconds which is approximately the total circulation time in dogs in surgical shock, as determined by injection methods, it would similarly be reasonable to believe that acetylene, re-breathed by man, would return within 14 seconds or so which is the total circulation time in man determined by injection methods. Hence, in many subjects, the acetylene method could not be used with any hope of accuracy, for the reasons discussed above.

It is quite true that this objection to the method would not apply to determinations in those subjects whose total circulation time is greater than 23 seconds. However, the determination of cardiac output by the acetylene method, having previously determined the total circulation time by an injection method, in order to be sure that it is not less than 23 seconds,

is hardly practical. In the second place, physiological variations within the same individual might easily allow the subject, at one time, to have a circulation time of greater than 23 seconds, and a few minutes later, to have a figure less than 23 seconds. This applies particularly under the conditions of the acetylene experiment since the subject is required to re-breathe violently enough to secure mixture and as a result increase his blood flow as much as 30 per cent (1). This increased blood flow must decrease the circulation time.

Another series of experiments bearing upon this question has been reported from the Hopkins laboratory (1, 9). When the acetylene samples are taken after a short period of re-breathing, the cardiac output figure is from 20 to 50 per cent higher than if the subject re-breathes a few seconds longer (1, 9). This is attributed to incomplete mixing on the basis (9) that complete mixing occurs only after 15 seconds re-breathing (18 seconds recumbent) which seems to be well attested (10). That the slight imperfections of mixing a few seconds earlier can produce the astonishingly large "errors" reported is rather a different question. In fact it would seem that imperfect mixing would act to reduce rather than to raise the putput for the following reasons. At the beginning and until mixing is complete there is more acetylene in the bag than the alveolar air. From the original description of the method (cf. (10) p. 111 and (1) p. 118) the samples would seem to be mostly alveolar air. If this is so, the second sample would be increased, relative to the first by any mixing which might occur after the first sample had been taken. This increase in the relative strength of the second sample would decrease the acetylene difference figure and hence the cardiac output.

If the samples are taken during inspiration and are composed of "bag" air rather than alveolar, we have another interesting consideration in the fact that the absolute value of the difference between the first and second samples would, other things being equal, be greater, the more soluble the gas. The rôle of imperfect mixing (taking as it does, under similar circumstances, a given amount of gas from the bag during the interval between the samples) would therefore be less important, i.e., would work a lesser change in the output figure. Now acetylene is more soluble in blood than either nitrous oxide or ethylene but there is nothing in the writings of Grollman to indicate that "imperfect mixture" does not play the same rôle whichever gas is used.

A further point that is brought out in one of Grollman's more recent papers (11) is that there may be complete acetylene equilibrium between lung air and *simultaneously* (sic.) obtained arterial samples five seconds after the subject begins to breathe the gas. The statement that agreement obtained in acetylene tension between alveolar air and arterial blood samples, each taken five seconds after the inhalation of acetylene, would

imply the author's belief that the equilibrium was reached earlier by two to four seconds (the lung to arm circulation time). The implications of such a belief are rather far reaching and should, of course, be substantiated by more critical evidence. They certainly lend at least dialectic support to the contention in the above paragraphs that short exposure periods need not, through inadequate mixture, lead to false high cardiac output figures.

The contention that there is a "plateau" in the cardiac output figure, lasting between 15 and 25 seconds' re-breathing time (1, 9) may or may not be confirmed in other laboratories. We did not think it worth while to repeat the experiments because in the light of our work such a plateau might be expected; a plateau whose significance can be interpreted in a wholly different fashion from the interpretation of the authors.

There would seem to be no difficulties in the way of assuming that the output figures are constant when samples are taken during the period of first return, are higher during an earlier period and are lower during the period of second and third return. The constancy of the putput figures during the first return is no criterion of their accuracy since they are calculated with no regard for venous acetylene.

The most important evidence in favor of the acetylene method is the reported agreement between the cardiac output as determined by the method in question and by the direct Fick procedure in man. A short series of experiments, when they bear internal evidence of variability, is not statistically adequate for conclusions on so important a matter. This is amply illustrated by experiments reported from the Louisville laboratories, showing agreement between the Fick outputs and the injection-method outputs (12), in spite of the fact that later work (3) has brought to light a systematic error of ten to fifteen per cent in the earlier technique. The same can be said of the ethyl iodide method (13) where the Fick checks are very close, though the earlier technique is not acceptable in the minds of most observers.

The agreement between the acetylene and Fick procedures reported by Grollman is no doubt in part due to the compensation of the error produced by re-circulation of acetylene tending to reduce the output figure by a similarly produced but opposite error due to the re-circulation of oxygen. That the errors compensate fairly well, "fortuitously of course" (cf. (2) p. 442), in these experiments is no indication that they may be expected to do so at all times and under all conditions.

SUMMARY

An attempt is made to evaluate the acetylene method of determining the cardiac output. It is suggested that some of the assumptions upon which the procedure is based, are contradicted by evidence derived from the literature and from experiments reported herewith.

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CONTRACTION AND EVACUATION OF THE GALL BLADDER OF THE RABBIT PRODUCED BY CHOLECYSTOKININ

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There is evidence indicating that the gall bladder of the rabbit may not contract and evacuate via the cystic duct. Halpert and Hanke (1929), by studying the concentration of methylene blue in hepatic and gall-bladder bile at certain periods after the administration of the dye, concluded that "in the rabbit bile does not under ordinary conditions leave the gall bladder through the cystic duct." Using the same method in the dog, Rewbridge, Hanke and Halpert (1930) concluded that exchange of gall-bladder contents occurs within about twenty-four hours. Lueth, Ivy and Kloster (1930) were unable to record gall-bladder contractions in the rabbit after injecting cholecystokinin by using the ordinary manometer or tamour methods.

Since Drewyer and Ivy (1930) found an appreciable quantity of cholecystokinin in the intestinal mucosa of the rabbit, it was considered worth while to use other methods for ascertaining whether the gall bladder of this animal may contract and evacuate via the cystic duct. We could not use the method of Graham and Cole (1925) because the gall bladder of the rabbit does not visualize well with sodium tetraiodophenolphthalein (Lueth and Ivy, 1929).

Methods and results. In two rabbits, 20 mgm. methylene blue per kilo of body weight were injected intravenously fifteen hours previous to the experiment. Halpert and Hanke (1929) have shown that this dye is concentrated in the rabbit's gall bladder and outlined a procedure for determining the concentration of the dye in bile, which was used in these experiments. In one experiment, the bile emitted was collected from washings of the duodenal loop into which the common duct drained. In a second experiment the bile was collected from the common bile duct by an indwelling cannula. It was found that after the injection of cholecystokinin, the total amount of methylene blue collected was about four times that collected during the control period of similar duration. Twenty-three thousandths milligram was collected in the control period and 0.077 mgm. in the period following injection of cholecystokinin. The gall blad-

der contained 0.006 mgm. methylene blue at the end of the experiment. In the second experiment, 0.27 mgm. of methylene blue was collected during a forty-five minute control period in a concentration of about 1–20,000. After 2.0 mgm. cholecystokinin intravenously (1.0 mgm., dog dose), 1.08 mgm. methylene blue in a concentration at first of 1–4500 and at the end of 1–11,700 was collected in forty-five minutes. Only a negligible trace remained in the gall bladder at the end of the experiment. In both experiments the bile collected was darker after cholecystokinin, the gall bladder became smaller and the blood vessels tortuous. Contraction rings were

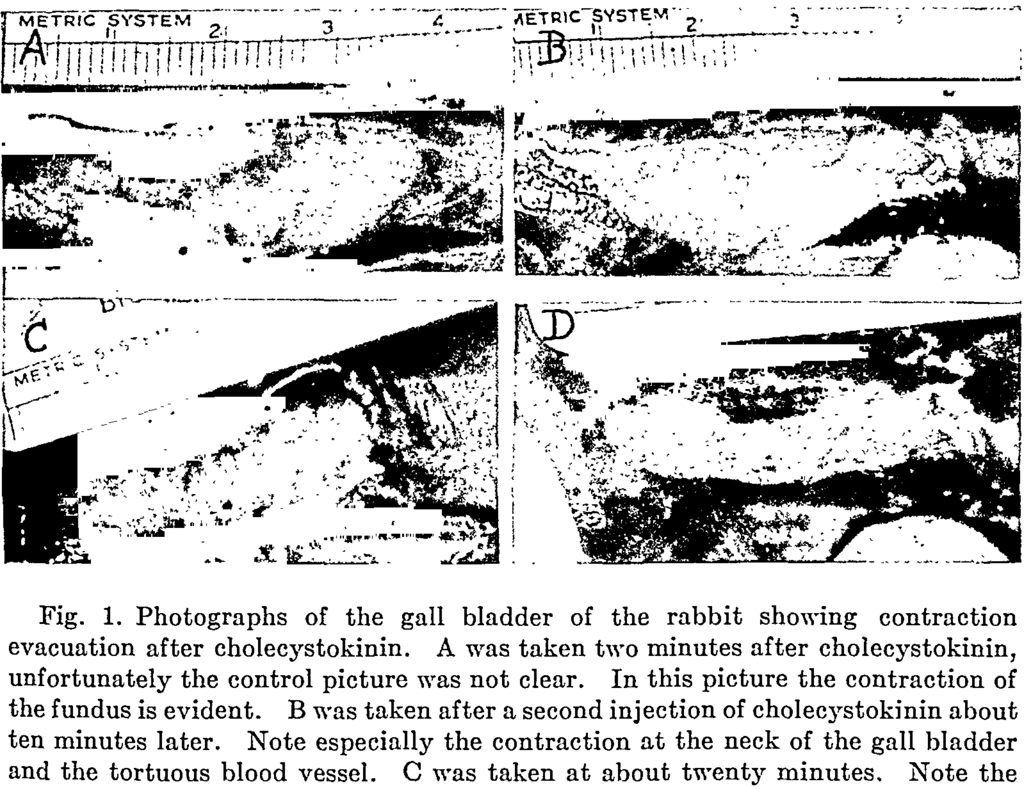

Fig. 1. Photographs of the gall bladder of the rabbit showing contraction evacuation after cholecystokinin. A was taken two minutes after cholecystokinin, unfortunately the control picture was not clear. In this picture the contraction of the fundus is evident. B was taken after a second injection of cholecystokinin about ten minutes later. Note especially the contraction at the neck of the gall bladder and the tortuous blood vessel. C was taken at about twenty minutes. Note the contraction ring. D was taken at about forty minutes after a fourth dose of cholecystokinin.

visible on the gall bladder and at the end of these experiments the gall bladder was contracted to a cord-like structure. The contractions of the gall bladder were demonstrated to various members of our laboratory.

In two other rabbits, photographs of the contracting gall bladder were made. Under ether narcosis, the rabbit's gall bladder was exposed and photographs were taken before and at intervals following the intravenous injection of cholecystokinin. The gall bladder was seen to contract as a whole, and also to have band-like contractions about the mid-region and cystic-duct area which appeared to progress toward the cystic duct. In

other instances, the contractions might start at the cystic duct and pass in a reverse direction over the gall bladder, or they might start at both ends and meet at the center. The contractions were not regular in regard to site of origin or direction of travel. The gall bladder was about one-third to one-half the original size at the conclusion of these experiments (see fig. 1).

In four other rabbits under ether anesthesia, the gall bladder was exposed, its bile aspirated and an equivalent amount of brominated oil was injected into the gall bladder, the site of needle puncture was tied off, the abdomen closed and the animals followed by fluoroscopy, x-ray photographs being made from time to time. In two rabbits, 40 per cent "brominol light" emulsion in acacia (Abbott) was used. Evidence of a small decrease in the size of the gall-bladder shadow was obtained in both cases after cholecystokinin. In two other rabbits "brominol light" (Abbott) was used as a contrast medium. After cholecystokinin, a change in contour of the gall bladder and a slight diminution in size was noted. After four days the brominol shadow was still visible and cholecystokinin gave a further slight reduction in the size of the shadow. We believe the viscosity of the brominated oil is too great for ready evacuation from the rabbit's gall bladder.

SUMMARY

The gall bladder of the rabbit has been seen by direct vision to contract and evacuate via the cystic duct under the influence of cholecystokinin and photographs have been made. The gall bladder of the rabbit does not contract with sufficient force to evacuate the larger portion of a viscous brominated oil placed within its lumen artificially, which is known to be evacuated in the cat and dog.

I desire to express to Dr. A. C. Ivy my appreciation for his many helpful suggestions during the course of this problem.

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THE CALORIGENIC ACTION OF LECITHIN

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The rôle of lecithin in body metabolism is still obscure. Bloor (1915) has observed that the lecithin content of blood may increase 20–30 per cent during the absorption of fat, hence the possibility that lecithin is an intermediate in fat oxidation. If this is the case, lecithin, given orally or intravenously, should be oxidized at least as rapidly as neutral fat and should exert a comparable specific dynamic action. Experiments were therefore planned to test this possibility.

Procedure. Hourly determinations of metabolic rate were carried out on three different dogs whose basal metabolic rate was determined from time to time. The dogs used were trained for 4 to 6 weeks prior to the experiments and were maintained at a practically constant weight on a diet of 75 grams of beef heart, 75 grams of cracker meal, 12 grams of lard and 5 grams of bone ash.

A closed circuit unit of the Benedict-Holmes (1912) type was used and all experiments were conducted in a room maintained at a constant temperature of approximately 25°C. Alcohol checks were repeatedly made. Bodily movements and in some experiments respiratory movements of the animal were recorded by tambour systems on a smoked drum.

After satisfactory basal controls had been established on preceding days or on the same day lecithin was administered either orally or intravenously. The lecithin, approximately 90 per cent pure, was made from beef spinal cord or from egg yolk. (Sixty to 90 grams of lecithin were usually given by mouth.) Three to four hundred cubic centimeters of a water emulsion, made with the aid of a mechanical stirrer and heated to 38°C., were given by stomach tube. Loose stools were generally observed on the day following, but vomiting, diarrhea and restlessness never occurred during the period that the animals were in the metabolism chamber. The metabolic rate in these animals was followed for 7 to 8 hours.

For intravenous administration, emulsions of lecithin, purified according to the method of Bloor (1929) were prepared in 100 cc. of 0.65 per cent sodium chloride solution. This concentration of salt was used since it produced a more stable and finer emulsion than did physiological saline.

Microscopic examination showed the lecithin particles to be all less than 7μ and the majority 1 to 3μ in diameter. The emulsion, kept in a water bath at approximately 50°C., was introduced through a needle into a saphenous or external jugular vein by gravity or under air pressure, the temperature of the entering solution 2 inches from the needle, ranging from 37.5°–40°C., depending on the rate of flow. The usual rate of infusion was 7 to 10 cc. per minute. Introduced in this way as much as 1 gram of lecithin per kilo was tolerated by animals without any obvious effect. Such doses could be repeated every day without apparent harm.

TABLE 1

Oral administration of lecithin

DOG	DATE	CONDITION	NON-PROTEIN R.Q.	URINARY N	CALORIES	PER CENT INCREASE OVER CALORIES GIVEN
				mgm./hr.	*per hour*	
4	4–17–31	Basal	0.83	104	11.73	
	4–17–31	Lecithin 70 gm.	0.83	142	11.58	0
	9–28–31	Basal	0.80	83	14.11	
	9–28–31	50 gm. lard	0.77	92	15.71	2.9
5	5– 1–31	Basal	0.83	62	13.98	
	5– 1–31	Lecithin 90 gm.	0.84	81	15.01	1.9
	9–25–31	Basal	0.91	139	14.05	
	9–25–31	Olive oil 55 gm.	0.79	136	16.45	4.9

Alcohol checks 0.669 ± 1.1. High –0.681. Low –0.657

The experiments in which lecithin was given intravenously were controlled by observing the effects of introducing equivalent volumes of 0.65 and 0.9 per cent saline solution on intervening days.

RESULTS. The data of table 1 illustrate the metabolic changes following oral administration of lecithin in comparison with those after feeding approximately iso-caloric quantities of neutral fat to the same animals. Comparison with basal determinations show that the non-protein R.Q. is unaffected by lecithin. The urinary nitrogen was substantially increased. In dog 4 which had received 70 grams of lecithin by mouth or approximately 150 per cent of the 24 hour basal caloric requirement, the heat production was unaffected. Dog 5, after 90 grams, showed a rise in metabolic rate, averaging through the sixth hour 15.01 calories per hour or an increase of 7.4 per cent. In this dog all doses (60–90 grams) exerted a small specific dynamic action, the average for all being 1.9 per cent of the calories

given. As shown in figure 1 the peak of the heat production is reached in the sixth hour. This specific dynamic effect is small as compared with the figure of Murlin and Lusk (1915) for neutral fat (4.1 per cent) or with values obtained on the same dogs in this investigation after feeding iso-caloric quantities of neutral fat (table 1 or fig. 1). Furthermore, the latter values were associated with a considerable depression of the respiratory quotient.

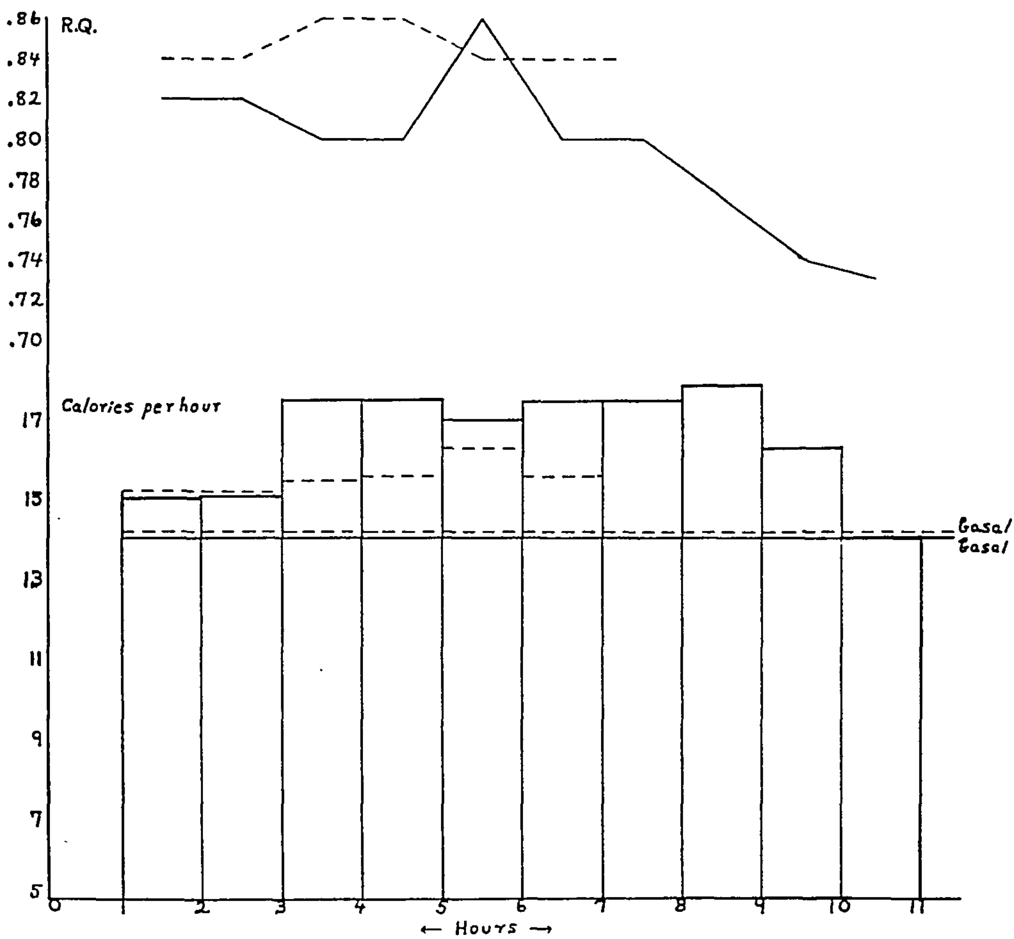

Fig. 1. Dog 5. Hourly changes in heat production and non-protein R.Q. after feeding approximately iso-caloric amounts of neutral fat (heavy line) and lecithin (dotted line).

These and other experiments indicate that lecithin per os has a much less dynamic effect than iso-caloric amounts of neutral fat and in some animals may exert no effect whatsoever. The first possibility that suggests itself for explaining the small or negative effect of lecithin is that it is not readily absorbed. To test this possibility intravenous introduction under controlled conditions was necessary.

Before discussing these results it is necessary to consider briefly the effects of saline infusions themselves. The results on two dogs are included in table 2. The basal determinations, 7 for each dog, show good agreement varying by ±1.3 and ±1.5 per cent respectively. The alcohol checks average 0.665 with a mean variation of ±1.1 per cent. The heat production followed for 5 hours after injection showed interesting

TABLE 2

Intravenous administration of lecithin

DOG	CONDITION	NON-PROTEIN R.Q.	URINARY N	CALORIES PER HOUR			PER CENT INCREASE OVER BASAL
				Average	High	Low	
			mgm./hr.				
	Basals	0.85	89	13.95	14.23	13.63	
	100 cc. 0.65% NaCl	0.92	105	14.87	15.59	14.30	+6.5
	Lecithin 100 cc. emulsion						
5	3 gm.	0.94	120	13.96	13.96		0
	5 gm.	0.80	108	14.50	14.54	14.44	+3.8
	9 gm.	0.81	112	15.01	15.23	14.83	+7.5
	Average	0.85	113	14.49	15.23	13.96	+3.9
	Basals	0.85	91	12.84	13.07	12.49	
	100 cc. 0.65% NaCl	0.90	125	12.59	—	—	−1.9
	Lecithin 100 cc. emulsion						
	3 gm.	0.97	130	13.19	13.53	12.85	0
	5 gm.	0.86	120	12.46	—	—	0
	9 gm.	0.89	124	12.34	—	—	0
	Average	0.91	125	12.67	13.53	12.34	0
	*Basal	0.84	110	12.49			
6	5 gm. lecithin	0.86	120	12.46			0
	*Basal	0.86	95	12.81			
	18 gm. lecithin	0.99	—	19.61			+53.0
	Alcohol checks	0.665			0.679	0.651	
			Murlin and Riche				
	Basals	0.80		14.61			
	Lard 3 gm.	0.75		15.33			+7.0

* Basal and experiment immediately below performed on the same day.

results. It varied in different dogs but was constant in the same animal. For instance, in two experiments on dog 6, injection of 100 cc. of 0.65 per cent salt solution did not modify the heat production but in four experiments on dog 5 the heat production was raised an average of 6.5 per cent. The increased heat production following intravenous infusion of saline was associated with the introduction of fluid; mere insertion of a needle and

conduction of the routine manipulations were found to have no such effect. The dogs displayed no nervousness or movements in the metabolism chamber which could account for the effect observed. The hypotonicity of the solution could also be ruled out since injection of 0.9 per cent saline gave similar results in the same dogs. Saline infusion also increased the nitrogen excretion by 22 per cent and elevated the R.Q. by an average of 0.06.

While the explanation of these facts is not clear, the possibility that intravenous injections of considerable quantities of saline may have a calorigenic effect in some dogs deserves emphasis, because it is so generally disregarded in evaluating metabolic data from experiments in which substances are given intravenously.

A survey of the literature shows that Wilhemj, Bollman and Mann (1931) reported an increase in heat production of approximately 30 per cent following intravenous use of hypertonic solutions of sodium chloride; however, 50 cc. of 0.9 per cent solution of salt had no such effect. An examination of their charts indicates that physiological saline may elevate the basal metabolic rate by 5 per cent; a figure only a trifle less than that found in my experiments.

Observations such as these make it important to determine the degree of susceptibility of each animal to saline infusion before attempting to interpret the effect of intravenous doses of lecithin. Exercising this precaution, the effect of lecithin, given intravenously, is noteworthy in its negativity.

The data of table 2 illustrate typical responses. In dog 5 the caloric output was increased on an average 4.6 per cent (5 experiments) as a result of intravenous doses of 3 to 9 grams of lecithin. The largest increase (7.5 per cent) occurred after giving 9 grams, but was only a trifle larger than the average due to salt solution (6.5 per cent).

In dog 6, three to nine grams of lecithin intravenously caused no discernible change in heat production, the percentage change ranging from 0 to −1.4 per cent. These slight or negative dynamic effects are still more striking when we consider that the larger doses (9 grams) are sufficient to increase the blood lecithin approximately 700 per cent. The non-protein respiratory quotients also gave no indication that lecithin is oxidized when a state of artificial lecithinemia is produced. In both dogs the quotient increased greatly after the injection of 3 grams. In dog 6 it also rose slightly after larger doses; in dog 5 it fell slightly below the basal level. A glance at the figures for hourly nitrogen excretion also shows no greater deviations than those following saline injection alone.

These effects are in striking contrast to the observations of Murlin and Riche (1915) who found approximately a 7 per cent increase in heat production, together with a decrease in the respiratory quotient after in-

travenous injection of 3 grams of emulsified fat. In view of the observations reported here, the question may be raised whether the increased heat production might not be attributed to an effect of the saline used, but this would not explain the decrease in respiratory quotient found.

Several attempts were made to increase the amount of lecithin injected beyond 1 gram per kilo. For example, dog 6 (table 2), was finally given double this dose (18 grams). The data show that the average heat production was increased 53 per cent over the basal, but the R.Q. increased to 0.99. The maximum heat production occurred during the third and fourth hour when a 75 per cent increase was noted. Such experiments could no longer be considered physiological for the animal later developed hemoglobinemia; the temperature rose to 40°C. for a few hours, paralysis of the legs developed and the animal died. Since lecithin is a hemolytic agent the caloric effect and attendant fever were probably due to a toxic action of lecithin rather than to its oxidation.

On the basis of all these results obtained in an investigation extending over a year it is concluded that lecithin introduced into the gastro-intestinal tract or directly into the blood stream exerts little or no specific dynamic effect, does not reduce the respiratory quotient and when given intravenously has no effect on nitrogen excretion by the kidney. The corollary logically follows that lecithin either plays no part as an intermediary in fat oxidation or that it is burned so slowly that it escapes detection. This also fits in with the observation of Nerking (1909) and Bloor (1914) that injected lecithin remains in the blood stream for many hours, whereas fat similarly injected is quickly removed (15 minutes) by the tissues. The body apparently does not have facilities for the removal of large excess of lecithin, either by storage or combustion.

SUMMARY

1. The possibility that lecithin is an interemdiary in the metabolism of fat was studied on dogs by determining the changes in heat production, in the R.Q. and in urinary nitrogen after feeding lecithin and injecting it intravenously.

2. In addition to control of the apparatus through alcohol checks and of the animal through repeated determinations of its basal metabolism, the effect of saline injections *per se* was taken into account when lecithin emulsions were administered intravenously.

3. The results show that some animals respond to intravenous injections of saline by increased nitrogen excretion and a slight augmentation of heat production which cannot be overlooked in evaluating the effects of lecithin injections.

4. Results indicate that lecithin given *per os* has a specific dynamic effect much less than an iso-caloric amount of neutral fat and that when

given intravenously in amounts up to 1 gram per kilo has no calorigenic effect at all.

5. These metabolic studies therefore offer but little support for the view that lecithin is concerned in the intermediary metabolism of fat or that it is an available source of material for oxidation.

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STUDIES ON HUMAN BLOOD PRESSURE CRITERIA AND METHODS

I. The Effects of Partial and Complete Occlusion on Actual Pressures in Compressed Arteries

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Statement of problem. How accurately blood pressure, say in a brachial artery, can be determined by existing criteria and apparatus is a debatable question. Assuming this possible, however, the larger question remains whether constriction or occlusion of a large artery affects the pressure in its proximal end to such an extent that even correct readings do not correspond to pressures existing in the aorta.

Gladstone (1929) concluded that systolic pressure readings can be fairly estimated as 14 mm. too high owing to conversion of kinetic to pressure energy and to water hammer action. More recently, O. Frank and Wezler (1931) voiced the opinion that through summation of reflected waves from a point of occlusion, the systolic pressure may increase 31 to 34 per cent.

Conclusive experimental evidence on this question appeared to be lacking however; hence this investigation.

Procedure. Our experiments necessitated finding a suitable artery with two side branches between which compression could be applied. By inserting an optically recording manometer into these two branches the changes of pressure in the proximal and central ends could be compared previous to and during various stages of compression and decompression. For this purpose a peripheral artery such as the femoral is unsuited because the pressure variations in the brachial artery of man retain many characteristics of the central pulse and are not modified by damping and reflected waves, as is the pulse in the femoral artery.

We finally selected for our work a portion of the left subclavian artery between the origins of the left internal mammary and deep cervical arteries, believing it better to utilize a vessel more central rather than one more peripheral than the brachial vessel. Small branches peripheral and central to these vessels were ligated.

Experiments were performed on anesthetized dogs whose chests were opened under properly controlled artificial respiration. After cannulae of two optical manometers were inserted in the internal mammary and deep cervical arteries and the manometers firmly fixed, a small metal clamp was applied on the subclavian artery between the two cannulae. The clamp had a lower stationary arm upon which the vessel rested and an upper movable arm for compression. By means of a fine screw arrangement extending through a fixed handle the degree of compression could be nicely graded. In this way a 3 mm. section of artery could be compressed or decompressed progressively or stepwise. It should be noted that a rigid compressor maintains a constant size of opening during systole and diastole and does not permit fluctuations such as occur when pneumatic compression is used. This has the advantage of simplicity and limits the physical variables to one, although as we later recognized it may modify conclusions as to diastolic pressure changes produced by compression.

Results. Figure 1 illustrates the pressure pulse changes which gradual compression produces in the distal (upper curve) and proximal (lower curve) portions of the vessels. They will be designated as proximal and distal pressure pulses hereafter.

The distal pressure pulse. The consecutive alterations in the distal pressure curve consist of a progressive decrease in amplitude, a slight accentuation of the primary peak, b', followed by its lower position on the anacrotic limb, a more gradual rise of each curve to a summit, c', a relatively higher position of the incisural notch, d', and a more sustained pressure after the notch, e'. The detailed changes, somewhat better shown in the insert at the upper right-hand corner, are typical of stenosis produced anywhere in the central vessels (cf. also records published by Katz, Ralli and Cheer, 1928).

The decrease in amplitude is chiefly occasioned by a fall in systolic pressure. As a rule the diastolic pressure likewise falls, though to a lesser extent. When compression is nearly complete, distal pressure always falls as a straight line, *D–E*.

The alterations during decompression are essentially the reverse, as is shown in figure 2. Several points warrant brief discussion, however. Between *A* and *B* the vessel was slightly decompressed and the small opening created was left unchanged until *C*, after which there was further progressive decompression as indicated by the upper line in the record. Between *B* and *C* each pulse beat forces a small amount of blood through the small orifice with the result that small pressure pulses occur with each cycle. Attention is called to the fact that the pressure variations, though greatly damped, are transmitted distal to the obstruction throughout the phase of systole, not merely during the initial steep rise of proximal pressures. As the lumen increases, *C–D*, the amplitude of the pressure curve

becomes greater and its form changes in reverse of that described as characteristic during compression.

The proximal pulse. The first essential change in the proximal pulse curve on compression (fig. 1) consists of an increase in the amplitude of the primary oscillation, *b*. Beginning at a time when a large pressure pulse still obtains distal to compression, *B–C*, this effect increases steadily until with nearly complete occlusion, *D*, it reaches a high peak and is followed by a series of decremental vibrations. These distort the summit, *c*, and doubtless contribute to its elevation. In relation to the normal true systolic pressures at *A* (160 mm. Hg) it was calculated that the peak of the primary oscillation, *b*, at *E* increases 25 per cent and the true systolic summit, *c*, rises about 11 per cent. On decompression the reverse changes obtain (cf. fig. 2).

A consideration of diastolic pressures shows (see proximal curves, figure 1) that it rises gradually after *D* and increases significantly from *D* to *E*, at which point an increase of about 15 per cent over that at *A* (94 mm.) was calculated. Such changes were the rule but to these there were exceptions, to which we shall allude later.

We conclude 1, that occlusion of a large artery intensifies the initial vibration, thereby momentarily raising the systolic pressure very considerably, 2, that, partly through superimposed after-vibrations, partly perhaps from other causes, the true systolic summit in mid-systole is increased, and 3, that as a rule the diastolic pressure is also elevated somewhat when occlusion is complete. Experimental observations therefore support *a priori* assumptions that complete occlusion of an artery can elevate pressures considerably above those normal for an animal or human subject.

Physical factors concerned. Gladstone (1929) has explained the elevation of systolic pressure in a compressed vessel above its normal level in accordance with generally recognized hydraulic principles (fig. 4). If liquid under a pressure head *P* flows from a reservoir A through a tube and stopcock *C*, the pressure in a side tube *B* reaches a lower level at *R*. The difference, *P–R*, represents the velocity head. If the stopcock is closed slowly liquid in *B* will rise to *P*, i.e., the kinetic energy of flow is converted

Fig. 1. Distal (upper) and proximal (lower) pressure curves from a subclavian artery during progressive compression beginning shortly after *A*. Time $\frac{1}{5}$ second. Details discussed in text. Insert shows form changes of distal pulse with greater amplitudes. About $\frac{1}{2}$ actual size.

Fig. 2. Distal, *2*, and proximal, *3*, pressure curves from a subclavian artery during decompression. Rate of decompression indicated by *1*. Time $\frac{1}{5}$ second. Discussion in text. About $\frac{1}{2}$ actual size.

Fig. 3. Distal and proximal pressure curves from subclavian artery during early stages of decompression, during slower heart rate, *E* segment showing normal pressure pulses. Time $\frac{1}{5}$ second. Reduction about $\frac{1}{2}$.

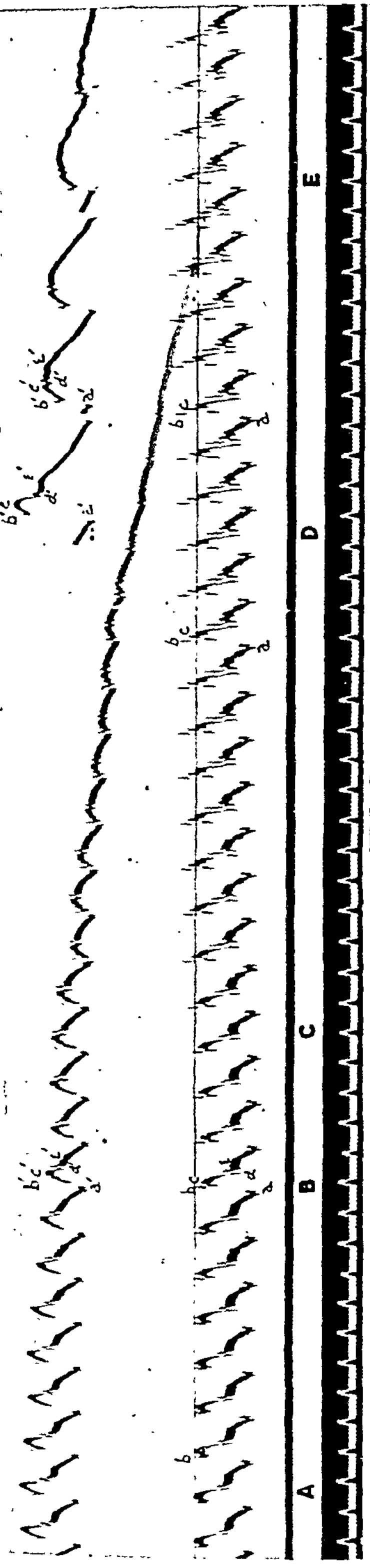

FIG. 1

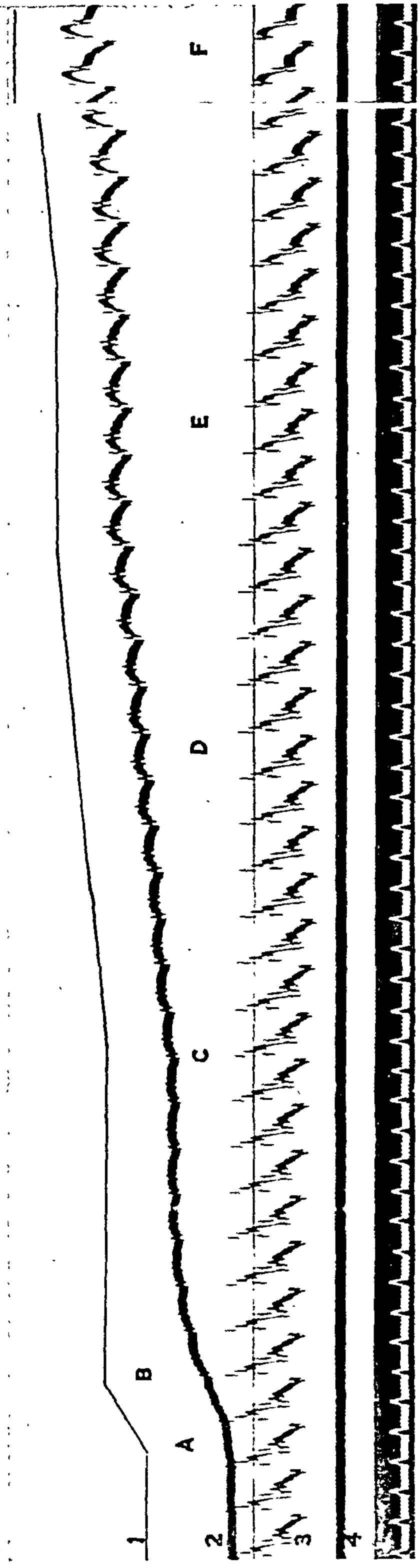

FIG. 2

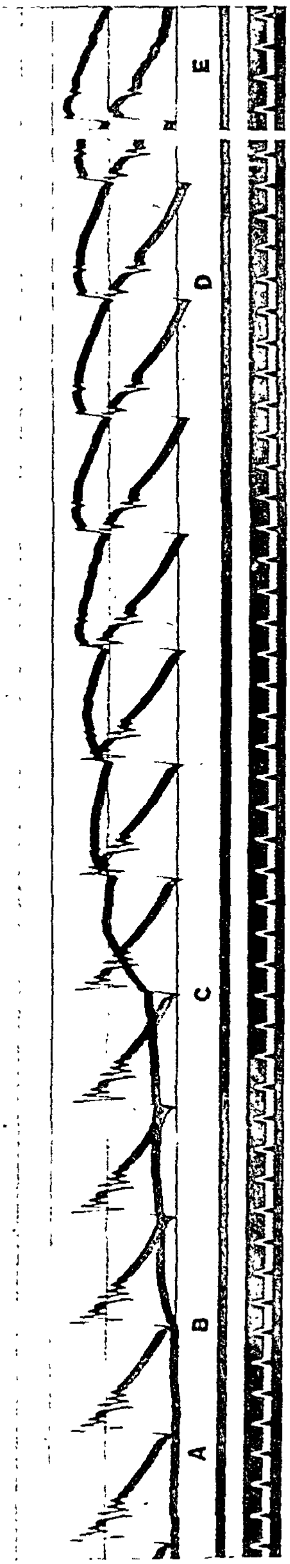

FIG. 3

into potential energy of pressure. If, however, the stopcock C is closed very abruptly the momentum of the onward moving column may cause a transient elevation of pressure in tube B to S. This is known as the "water hammer effect" and the increase of pressure above the total pressure head (i.e., P–S) is taken as a measure of the energy contributed by water hammer. On such hydraulic principles, Gladstone explains also the rise of pressure in an occluded artery: to the natural systolic pressures are added pressure energy due to conversion of kinetic energy of flow into potential energy of pressure and pressure energy due to water hammer.

It is obvious that such conceptions are based on hydraulic experiments in which constant pressures and constant flow obtain, in other words in which conditions are more nearly in dynamic equilibrium. In the blood vessels, however, pressure and flow both undergo pulsatile variations; they never approximate a dynamic equilibrium. Hence if we choose to

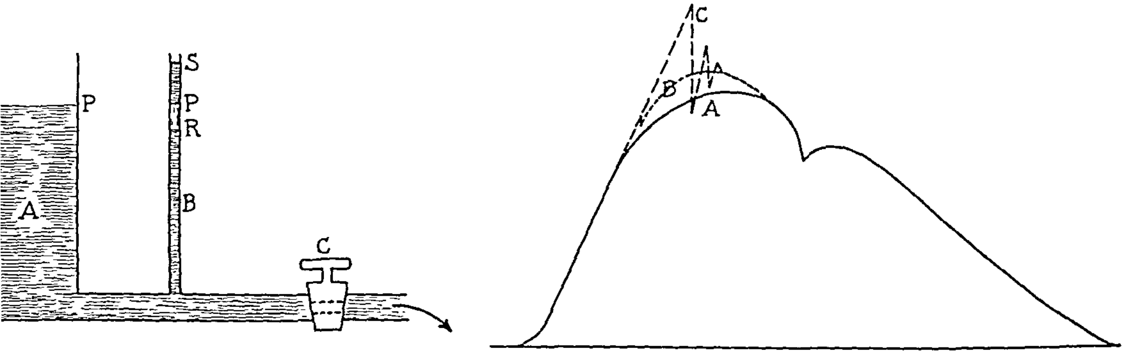

Fig. 4. Diagrams illustrating effects due to conversion of velocity to pressure head and water hammer in system in dynamic equilibrium (left) and the effects on pressure curves of arterial system not in dynamic equilibrium (right).

apply the conceptions of converted energy of flow and water hammer to dynamic changes in the circulation it is necessary to restate our conceptions. An attempt at doing so may be ventured. Let the curve A of figure 4 represent the natural pressure changes in an artery. The additional pressure due to conversion of velocity to pressure head upon occlusion of a vessel may then be represented by the curve B. The added effect due to water hammer which is transient in nature may be pictured by the superadded primary oscillation and its after-vibrations shown as curve C. According to such a conception, which also takes account of the vibratory character of water hammer, the temporary elevation of pressure during the primary peak in our records could be considered an effect of water hammer.

It is doubtful, however, whether the effects imputed to converted velocity head and water hammer can be safely dissociated in this manner. Since the velocity maximum according to Broemser (1928) always pre-

cedes the pressure maximum, it may contribute to the increased primary oscillation when a vessel is occluded. On the other hand, the after-oscillations superimposed on the summit and damped to a variable degree under different circulatory conditions (e.g., existing internal pressures, elasticity coefficient of vessels) may contribute to the actual elevation of true systolic pressures. With these difficulties before us, we should like to raise the question whether it is not advisable to abandon altogether the terms "water hammer" and "reconverted kinetic energy" in discussing circulatory dynamics. Certainly the term "water hammer" has tended to confuse rather than clarify many circulatory problems.

The complications involved are all obviated if pressure curves are analyzed by a combination of the "compression chamber" and "wave motion" theories developed by Frank (1898, 1905). According to this conception the elastic vessels are regarded as a compression chamber, storing energy of pressure during systole and converting it into kinetic energy of flow during diastole. The fundamental form, the amplitude and the actual systolic and diastolic pressures are determined by the character of the systolic ejection, by the degree of peripheral resistance and by the elasticity coefficient of the arterial walls. The fundamental characteristics so established are modified, however, (a) by waves reflected from obstructions or bifurcations of vessels, and (b) by natural vibrations set up simultaneously in the arterial walls and blood column whenever abrupt variations of pressure occur.

Applying this form of analysis to our experimental records, it is obvious from the great intensification of the primary oscillation and the introduction of decremental after-vibrations that the last mentioned factor (b) is the most important one affecting systolic pressure when an artery is occluded. Reflected waves may also enter to modify pressures as Frank believes, but our observations show that they seem to be of little importance in the region immediately proximal to compression. That they may under normal conditions affect systolic pressures in more central vessels is quite possible.

We therefore conclude that the elevation of systolic pressure in the vessel immediately proximal to an occlusion is most easily explained by the creation of oscillations which greatly intensify the primary peak momentarily and by superposition elevate the true systolic summit to a lesser extent.

Significance of results in estimation of blood pressures by clinical methods. The demonstration that systolic and diastolic pressures are mechanically increased proximal to obliteration does not *ipso facto* imply that readings of systolic and diastolic pressures by commonly used criteria are also correspondingly high. Such, however, has been the general inference. An inspection of records published by Erlanger (1921), Bramwell and

Hickson (1926) and Frank and Wezler (1931) clearly indicates that the first audition of sounds peripheral to a point of compression, as well as oscillatory and palpatory criteria of systolic pressure, requires the passage of at least a small pressure wave through the region of constriction. There are no *a priori* grounds for believing that the momentary pressure peak represented by the primary oscillation can accomplish this; and our experimental observations definitely preclude such a possibility. Thus, as is clearly seen in the portion of curve between *B* and *C*, figure 3, a pulse beat that penetrates a constriction is always accompanied by distal pressure variations throughout systole, and is in no case limited to the early moments of systolic ejection alone. Close inspection reveals similar phenomena in the smaller curves reproduced at *D* in figure 1 and at *A* in figure 2.

Further examination of our records shows that, although the primary oscillations continue to be intensified, the true systolic level (at *c*) in the proximal pressure curve is very quickly restored to normal after several pulse beats have passed beneath the compressor. Thus, in the curves of figure 2 this occurs at *C*, after 8 beats have passed and in the records of figure 3 it occurs after passage of 9 beats, i.e., at *D*. Unless, therefore, systolic pressures are measured almost immediately after the smallest waves traverse the de-occluded artery, which is improbable in practice, any slight elevation of true systolic pressure is probably of academic rather than practical importance.

Unfortunately our investigations allow only probable rather than quite certain conclusions regarding mechanical changes in diastolic pressure. The criteria for determining diastolic pressure are not so precise as those governing estimation of systolic pressure. We found it technically impossible also to register sound variations in conjunction with our other records. Finally compression by a rigid clamp, though favorable for evaluation of changes in systolic pressures, doubtless introduced difficulties as far as interpretation of diastolic pressure changes is concerned. In fact, it was not our intention originally to study these relations at all.

One deduction can, however, be made from these experiments. A glance at the proximal pressure curves shows that diastolic pressure remains constant and normal between *B* and *D* in figure 1 and between *C* and *D* in figure 2 except for slight respiratory variations which normally occur. While it is obviously impossible to fix definitely the exact place where diastolic pressure criteria would apply it will no doubt be agreed by all that it would be somewhere in this region of normal diastolic pressures. Hence, it is obvious that, while diastolic pressure is elevated when a vessel is nearly or completely occluded, it is not raised at the time when diastolic pressure criteria would apply. On the other hand, we have a number of records in which diastolic pressure decreased slightly during partial occlusion. This occurred particularly when hearts were slowed by vagus stimu-

lation and the arterial pressures had become stabilized at lower levels. The curves of figure 3 show such effects. We do not maintain that similar changes necessarily obtain when a vessel undergoes pneumatic compression, but we venture the conclusion that if partial compression affects diastolic pressure in the vessel at all, the modification is in the nature of a reduction rather than an increase.

Influence of abnormal states of circulation. In addition to studying the problem under circulatory conditions normal for the dog, we investigated it also during abnormal circulatory states; for example, when heart rates were very fast or very slow, during hyper- and hypo-tensive states and particularly during experimental aortic insufficiency. Unfortunately no way

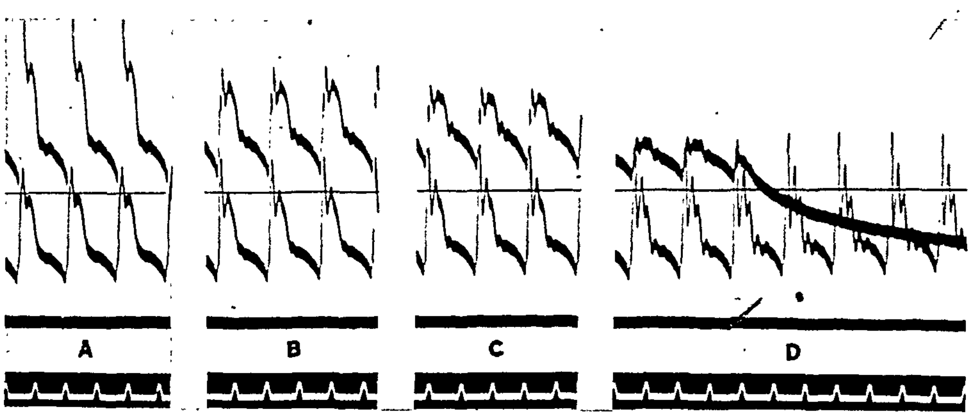

FIG. 5

Fig. 5. Four segments of distal (upper) and proximal (lower) pressure curves from subclavian artery during experimental aortic insufficiency, showing changes in contour during various stages of compression. A, normal uncompressed; B and C, partially compressed; D, nearly complete and completely compressed vessel. Time $\frac{1}{5}$ second. Reduced to about $\frac{1}{2}$ actual size.

was found to study the modifications introduced by altered elasticity of large vessels.

The effects were qualitatively alike under all conditions studied; the chief quantitative difference being the degree to which the primary oscillation was intensified. In general, the magnitude of its increase and also its period were inversely related to the height of diastolic pressure. For example, during hypertension produced by reflex vasoconstriction (central vagus stimulation) the primary oscillation was increased much less than in the illustrations accompanying this paper; its period was shorter and after-vibrations were highly damped. The reverse occurred when diastolic pressure was lowered in various ways.

Extreme lowering of diastolic pressure was produced by experimental

aortic insufficiency. The analysis of records obtained is interesting because the subclavian pressure curves show a characteristic intensified primary oscillation even without occlusion. Since the period of the oscillation is also relatively long the curves have a peculiar spiked appearance, a characteristic which is responsible for the so-called water hammer sensation on palpation. Segment A of figure 5 shows these normal characteristics in both proximal and distal pulses. Segments B and C illustrate the intensification of this phenomenon on progressive compression, and segment D the changes as compression becomes complete. The primary oscillation in the proximal pulse is now definitely followed by a large second vibration which gives the curve a typical bifurcated appearance. The pressure at the summit of each peak is greatly increased, being respectively 39 and 16 per cent above true systolic pressure in segment A. The distal pulse record in this segment shows, however, that even repetitive bombardment of a nearly occluded vessel by very high pressure waves of short duration is not sufficient to transmit a pressure wave distal to the compression. If this is true of a narrow region of compression (3 mm.) it is more certain when a greater length of artery is compressed by a pneumatic cuff.

We therefore conclude that the pressure rise generally attributed to water hammer even when extremely intense, as during aortic insufficiency, exerts little effect on the criteria by which systolic pressures are evaluated clinically and chiefly for the reason that the oscillations produced are so transitory. In closing the discussion we may state that these results and conclusions were entirely unexpected by us, for in common with others we were inclined to believe that mechanical factors might introduce considerable error, particularly in systolic pressure readings.

SUMMARY

The question was investigated experimentally whether constriction or occlusion of a large artery during estimation of human blood pressures elevates the pressures in its proximal end to such an extent that even exact pressure readings do not correspond to those in the aorta.

Pressures from side branches of a subclavian artery were optically recorded and the vessel was compressed between the points of registration.

The results show that true systolic pressure in the proximal end is elevated very little and sometimes not at all during complete occlusion; in any event it returns to that normal for the vessel soon after pulse waves pass the constriction. The primary oscillation of the pressure curve, however, is intensified and followed by decremental after-vibrations. The degree of intensification, the number of after-vibrations and their decrements are inverse functions of the height of diastolic pressure, hence they vary in different circulatory states.

The physical factors responsible for the elevation of systolic pressure during occlusion are discussed. An attempt is made to adapt the usual conceptions of a converted velocity head and water hammer to conditions obtaining in blood vessels. The suggestion is made, however, that these terms can to advantage be abandoned and replaced by an analysis based on a combination of the "compression chamber" and "wave motion" theories elaborated by Frank. In accordance with this analysis neither the fundamental form of the pressure pulses nor the height of true systolic pressure is significantly altered by compression of a vessel. Occlusion and constriction cause the walls and blood column to be thrown into more or less violent oscillations which mirror themselves in an intensified primary oscillation and decremental after-vibrations. The first precedes and the latter are superimposed on the summit of the fundamental curve. Reflected waves do not appear to modify the pressure immediately proximal to the point of compression.

Since true systolic pressure is not ordinarily elevated after pulses penetrate a constriction, since greatly intensified or even repeated oscillations are incapable of causing a pulsation distal to constriction and since auscultatory, oscillatory and palpatory criteria for determining systolic pressure all require the transmission of at least a small pulse through the constriction, we reach the conclusion that experimental evidence does not favor the view that systolic pressure readings obtained by clinically applicable criteria are necessarily too high. Experimental evidence is presented that this also holds when violent vibrations of arterial walls and blood column are set up, as in experimental aortic insufficiency.

Experimental evidence demonstrates that while diastolic pressure may be elevated during complete occlusion it is not above normal at a time when criteria for diastolic pressure would apply. Under certain conditions, however, diastolic pressure decreases, hence the conclusion that estimations of diastolic pressure by clinical methods may be too low, rather than too high.

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THE CIRCULATORY CHANGES DURING HYPERTHERMIA PRODUCED BY SHORT RADIO WAVES (RADIOTHERMIA)

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The use of artificial hyperthermia in the treatment of certain infections and their consequences has received a great impulse from the development of physical apparatus by which controllable hyperthermia can be produced. Two forms of apparatus are used, viz., that employing high frequency currents directly applied to the body by means of electrodes and commonly referred to as diathermy, and that by which short radio waves are caused to traverse the body. In order to differentiate between hyperthermias produced by these means, we shall refer to the latter as *radiothermia* without any implication that it necessarily differs from *diathermia.* The recent introduction of satisfactory apparatus for producing high temperatures in patients by passing short radio waves through the body (Carpenter and Page, 1930) necessitates an inquiry as to whether the circulatory reactions produced are similar in kind and degree to those caused by application of external heat or whether the passage of electromagnetic waves *per se* produces still other changes which are beneficial or deleterious.

In order to validate such comparisons, tests should be made on the same species of animals under approximately identical experimental conditions and by use of the same type of apparatus. Inasmuch as Cheer (1928), in close association with one of us, has studied the cardiac and dynamic effects of hyperthermia in dogs heated in a hot air cabinet, it seemed particularly appropriate to study the circulatory effects of radiothermia in this laboratory.

PREVIOUS WORK. The literature on the circulatory effects of *hyperthermia* produced by external heat is covered in the publication by Cheer (1928) and in the recent survey by Bazett (1931). The circulatory changes during radiothermia and diathermia have not been fundamentally investigated to our knowledge. However, several physiological consequences which may possibly affect the circulation indirectly have been reported:

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1. It is quite generally agreed that the CO_2 content of the blood is reduced and that a state of mild alkalosis occurs in all forms of hyperthermia as well as in many fevers (Koehler, 1923; Cajori et al., 1923; Nasset, Bishop and Warren, 1931; Nasset, 1931; Banus, 1928, 1931; Bischoff and associates, 1929, 1931). Such a state of acapnia and alkalosis may conceivably reduce venous return to the heart (Y. Henderson, 1923), thus decreasing the minute output and arterial pressures of the resting animal; although it remains to be demonstrated that this is not nicely compensated for mechanically by the increased pumping action of respiratory movements.

2. Several investigators believe they have demonstrated a decrease in circulating blood volume but no general agreement exists as to the types of hyperthermia in which such reduction obtains. Bischoff and his associates (1929, 1931) concluded on the basis of hemoglobin changes in man that reduced blood volumes occur only in radiothermia, thereby differing from hyperthermia caused by diathermy and application of external heat. McIntosh (1931) using the CO method on anesthetized rabbits concluded that diathermia causes a reduced blood volume independent of temperature changes, whereas pyrexia produced by vaccines and infra-red radiations increases it (McIntosh, Kajdi and Meeker, 1930). More convincing evidence must be supplied, however, before we can accept the postulate that the circulatory volume decreases in hyperthermia produced by passage of electromagnetic vibrations of high frequency through the body.

3. A number of investigators postulate a decreased oxygen carrying power of the blood due to the fact that a more stable or non-functional form of hemoglobin is formed (Koehler, 1923; Banus, 1928). The observations of Bischoff and associates (1929–1931) that venous blood contains more oxyhemoglobin during hyperthermias and particularly in radiothermia makes it unlikely that an *anoxic anoxemia* affects the circulatory picture. On the other hand, the observations of Nasset *et al.* (1931) which indicate that the great increase in basal metabolism cannot be accounted for entirely by the high temperatures make it possible that a tissue anoxemia could occur despite the high O_2 content of venous blood. It is obvious that with so many conflicting elements it is difficult to decide whether tissue anoxemia really occurs.

APPARATUS. A small model of the short wave generator (Radiotherm, Model 200) manufactured by the General Electric Co. was used to produce hyperthermia. It was constructed on the same general principle as the larger apparatus for human beings described briefly by Carpenter and Page (1930). The unit consists of a transformer, a rectifier and a vacuum tube oscillator. The following data were kindly furnished by the Vacuum Tube Engineers of the General Electric Co.:

"The set is operated from 110 volt, 60 cycle, A.C. mains and, at normal conditions of operation, draws a current of 6 amperes. The power input to the set is then approximately 600 watts.

An oil-immersed transformer having a 4500 volt secondary and feeding a full wave rectifier forms the 2000 volt D.C. anode supply for the oscillator. This transformer has separate windings operating the filaments of the rectifier and oscillator tubes. A rheostat is connected in the primary of the high voltage transformer to provide anode voltage regulation

The rectifier consists of two half-wave hot cathode, mercury vapour tubes. No filtering circuit is used as the high voltage need not be a pure D.C. potential.

A shunt-feed, push-pull circuit operating over a frequency range of 9000 to 12000 KC is used as the oscillator circuit. Best operation is obtained at about 10,000 KC. In this circuit the heater plates are in parallel with the oscillatory circuit coil and the high voltage is blocked from the heater plates by means of two 6000 volt condensers. This oscillator uses two FP-1 pliotrons and delivers 150 watts of high frequency power."

In order to send short radio waves through the body of an animal and still employ optical manometers for registration, a great deal of ingenuity had to be exercised in order to avoid interference between the physical equipments. The following arrangement was found suitable, however, for studying the changes of respiration and variations of central arterial pressures in the innominate artery by optical methods:

Small dogs, 8 to 10 kilos in weight, were anesthetized with a small dose of morphine and sodium barbital (intravenously). The animal was placed on a wooden animal board enclosed in a housing made of dry wood and with two partitions fore and aft. The former allowed the head and neck to protrude, the latter completely enclosed the hind quarters of the dog; but by raising this partition, rectal temperatures could be read and apparatus adjustments easily made. The heater plates connected to the radio frequency oscillator consisted of two aluminum plates which were suspended by glass rods from the top of the box and hung to each side of the animal. Vertical and horizontal adjustment of the plates was achieved by very simple devices.

A universal optical manometer was inserted into the carotid artery low in the neck so that the tip of its cannula reached the innominate artery, a technique prescribed by Frank as an essential to trustworthy registration of central pulses. Intrapleural pressures were recorded by inserting a glass cannula through an intercostal space and connecting this to a tambour. Electrocardiograms were taken in some experiments, but not in all, as this involved temporary interruption of the radiotherm in order to avoid damage to the string.

In the earlier experiments attempts were made to read temperatures in various portions of the body with the aid of thermocouples, but it was soon found that they could not be relied upon owing, apparently, to too much induction in the thermocouple leads. Earlier fears that mercury thermometers could not be used were fortunately found to be groundless by care-

ful physical tests which assured us that temperatures of surrounding fluids and tissues are actually recorded by mercury thermometers placed in contact with them. For example, thermometers placed in various solutions and conducting solid media always recorded, when currents were flowing, the same temperature as another thermometer inserted immediately after the current had been turned off. Similarly, thermometers inserted into the rectum, under the skin and into heart cavities via the jugular vein, remained at exactly the same temperature for many minutes after discontinuing radiothermy.

PROCEDURE. Experiments were performed on 21 dogs. The general procedure, from which there were certain variations for special purposes, was as follows: With the animal in position between the heater plates, control records of respiration and central arterial pressures were recorded optically and sufficiently often to assure ourselves of the existence of a stable state. Radiothermy was then started—short records being taken at 2 minute intervals for the first 10 or 12 minutes; after that, at 10 minute intervals until the temperature reached desired levels. Readings of rectal and heart temperatures were made after each of these records and the optical manometers were frequently calibrated under static conditions. This procedure enabled us to study any effects that radiothermy may have on circulation and respiration apart from the elevation of temperature. Further information on this point was also obtained by shutting off the current for intervals of 5 to 15 minutes after temperatures had been raised to different degrees; but this procedure was adopted in only a smaller proportion of experiments.

The initial temperature of dogs varied widely, depending largely on the season of the year. As experiments were performed during winter, spring and summer months, further opportunity was offered for evaluating the effects of currents before the temperature had reached 38°C.

EXPERIMENTAL RESULTS. The general course of events in animals whose initial temperature ranged from 37 to 37.5°C. is first described. Simultaneous readings of rectal and heart temperatures uniformly showed a gradual deviation, the rectal temperature increasing more rapidly than the heart temperature. When radiothermy is uninterrupted, plots of cardiac temperatures show a steady linear increase until 40 or 41°C. is reached. After this and generally coincident with an increase in respiratory rate and amplitude, the temperature rose more gradually, probably on account of the greater heat loss through respiration. Death occurred at various heart temperature levels. In a few animals it was as low as 42.1°C.; in others, not until 44.5°C. had been reached (cf. fig. 1).

As in hyperthermia due to external heat, the respiratory movements of dogs increased in amplitude and frequency. The intensity of the respiratory stimulation depends on the degree of anesthesia and particularly

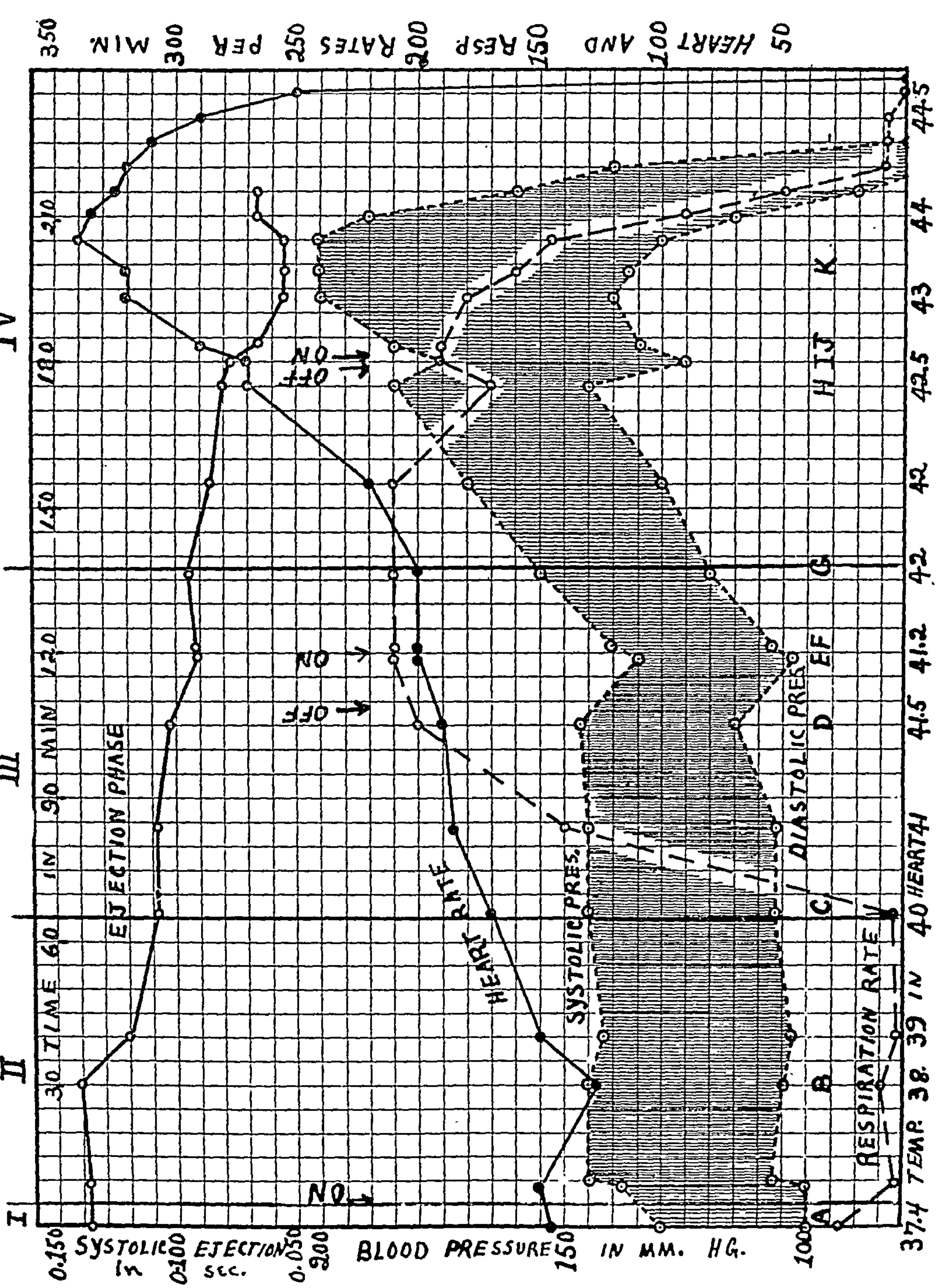

Fig. 1. Chart showing circulatory, respiratory and other data during radiothermia. Arrows "on" and "off" denote temporary suspension of radio waves. Letters *A*, *B*, *C*, etc., denote times at which records correspondingly labeled in figure 2 were taken. Discussion in text.

on the amount of morphine used, hence the ultimate depth and frequency encountered varied greatly when morphine was not used or employed in ineffective doses. Respiratory rates of 310 to 330 were not uncommon without morphine. With even moderate doses of morphine the acceleration, even at temperatures of 40 and 41°C., was not marked enough to prevent the occurrence of expiratory pauses. These different effects on respiration are mentioned as they must have a considerable effect in determining changes of blood CO_2 and blood alkali. Just before death the amplitude and frequency of respiratory movement decreased rapidly and respiratory failure occurred before cessation of the heart.

Electrocardiograms taken while radiothermy was temporarily shut off at various temperature levels showed no significant changes until critically high temperatures were reached. An abbreviation of the R-T interval and some decrease in voltage of the ventricular deflections were noted, however. The end effects were those of asphyxia following respiratory failure. No attempt was made to study these in detail, but it is of interest to note that fibrillation did not appear to be the ultimate cause of ventricular stoppage. Definite, discrete complexes of the ventricular type indicate that coördinate action was maintained to the last.

Changes in the heart and circulation. From optically recorded curves of pressures in the innominate artery it was possible to calculate changes in 1, heart rate; 2, duration of systolic ejection and occasionally isometric contraction; 3, the S/C ratio, and 4, systolic, diastolic and pulse pressures with reference to a calibration curve. Such data, plotted in relation to respiration rate, temperature and time, as in figure 1, give a general idea as to the course of events. In addition, optical pressure records taken at different intervals were carefully studied as to contour changes and their significance estimated in the light of other dynamic changes.

The chart of figure 1 illustrates the effects in an animal whose respiration was markedly stimulated and the photographic records of figure 2 are actual curves taken at corresponding letters on the chart. In this, as in most animals of our series, the vagi nerves were left intact. For convenience of description, the chart is divided into four sections (I to IV). The first (I) gives data before radiothermy was applied. The second (II) shows the events during the initial increase in temperature up to moderate levels, usually just below 40°C. This division is made, inasmuch as similar temperatures are frequently reached in normal dogs during the period of hot summer weather and hence may in a way be regarded as within the range of yearly normal for these fur-coated animals. During this stage, the respirations showed no significant increase in rate or depth, hence it is still possible to compare optical pressure pulses during expiratory rest (cf. segments A, B and C, fig. 2). The heart rate, except for a slight initial decrease at the temperature of 38°C. (B, figs. 1 and 2) accelerated slightly

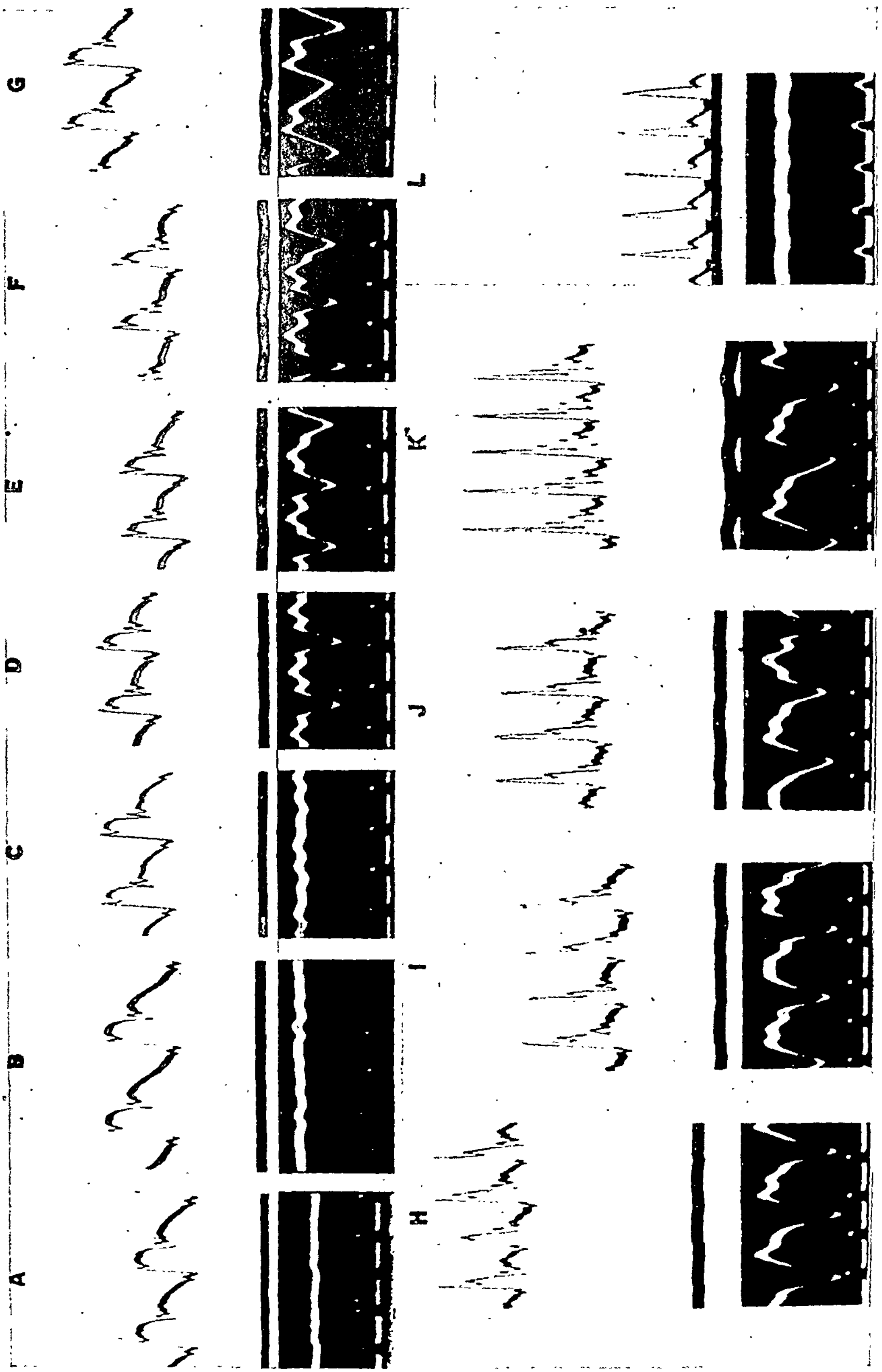

Fig. 2. Segments of optically recorded central pulses (upper) and respiration (lower) during progressive radiothermy. Segments E, F and I, J were taken during discontinuance of radiothermy. Letters A, B, C integrated with chart of figure 1. (Greatly reduced and slightly retouched for reproduction.) Discussion in text. Time $\frac{1}{5}$ second.

and showed a little decrease in the duration of systolic ejection. Pulse pressures measured during expiratory apnea were found to be increased a few minutes after the beginning of radiothermia, and, regardless of the initial slowing, diastolic pressure increased almost immediately. These were constant effects in all experiments. Once elevated, systolic and diastolic pressure showed little alteration until the end of this period. The optical curves themselves (fig. 2, A, B, C) display only minor changes in contour. A slight exaggeration of the primary oscillation, a greater drop of pressure after the incisura and a slightly more gradual decline of pressure during diastole are obvious in comparing segments A and C.

Applying the principles of arterial pressure curve analyses[2] we can conclude from the records that in addition to the slight cardiac acceleration the initial velocity of ventricular ejection is increased but we cannot be certain as to whether the total discharge during each systole was changed. The higher diastolic pressure can certainly not be attributed to increased heart rate alone, for it occurs also during the period of slight retardation.

The stage of moderate hyperthermia (III) is somewhat arbitrarily demarcated on the chart of figure 1 by temperatures of 40 and 42°C., because it represents a temperature range beyond which the therapeutic use would not be likely to go. During this stage the heart rate accelerated steadily, reaching a rate of 200 per minute at 42°C. Coincident with this, the ejection phase decreased from 0.11 to 0.09 second. Despite this great acceleration, the pulse pressure continued to increase, both systolic and diastolic pressures being elevated. These changes were progressive when radiothermy was not interrupted; they were temporarily held in abeyance, as in this experiment, during the period of discontinuance.

The effects on the pressure curves are illustrated by segments D and G, figure 2, those of segments E and F being recorded during temporary cessation of radiation. In segment D the pressure curves show, in addition to the elevation of diastolic pressure and reduction in pulse pressure, less steepness in the diastolic gradient. These changes indicate either that the run-off from the arterial system is decreased or that the elasticity coefficient of the arterial wall is increased. The curves of segment G show still greater increase in pressures and pulse amplitude, but the incisural fall of pressure and the diastolic gradient are both steeper in comparison with that of segment D. Applying the principles of curve analyses to these records the conclusion is reached that the hypertension and large pulse pressures are only partly due to cardiac acceleration and chiefly to a greater increase in initial velocity of ejection, though it must remain questionable in view of the greatly abbreviated period of ejection whether the volume of systolic discharge is increased.

[2] One of us (W) hopes to summarize fully the principles applied in this laboratory, in the near future.

During this stage of hyperthermia there is then first some indication of diminished efflux from the arterial system or an effect on the elasticity of arteries; but this is overcome toward the end of this period by greater elevation of arterial pressures due to increased cardiac activity.

During the fourth stage (fig. 1, IV) the respiratory and circulatory phenomena are intensified until the crisis is reached,—in this case, at a temperature of approximately 44°C. Respiratory movements increase tremendously, both in rate and amplitude, as does the heart rate which finally attains a frequency of 340 per minute at *K*. This is followed by the crisis; the respiration stops, the heart rate decreases and pressures sink rapidly. Up to the crisis, systolic and diastolic blood pressures continue to increase as does the pulse pressure. The pressure curves themselves (H and K, fig. 2) show that the systolic ejection phase is deformed through *an extreme intensification of the primary oscillation which is followed by a* distinct second oscillation. At the beginning of relaxation a very deep incisural fall of pressure occurs and there is very little additional drop during diastole. In other words, despite the extremely high pressures very little efflux from the arterial tree seems to occur during diastole.[3]

Discussion. If we compare the circulatory changes produced by radiothermy with those obtained after heating an animal by external means (cf. Cheer, 1928) certain similarities are obvious. The rapid increase in rate and depth of respiration and the primary failure of the respiratory center at extreme temperatures are similar. The initially gradual and subsequently more abrupt acceleration of the heart, also the characteristic abbreviation of systolic ejection are alike in both forms of hyperthermia. But hyperthermia from application of external heat is accompanied by progressive decrease in blood pressures and diminution of pulse pressures (Cheer, 1928); the pressure pulses acquire a peaked appearance and are devoid of all traces of primary oscillations, indicating reduced volume discharge, combined with decreased vigor of ventricular contractions. As already described, the changes produced by radiothermy are in many respects quite the opposite. The diastolic pressure increases, pulse pressures are greatly elevated and there is every evidence from the appearance of sharp primary oscillations that the vigor of ventricular contractions is increased to the last.

It is worth while digressing for a moment from the main theme of this paper since the experiments demonstrate remarkably well the importance of taking pulse form as well as numerical values for pulse pressure into account in drawing conclusions regarding systolic discharge. A glance at the numerical changes in systolic and diastolic pressures plotted during the

[3] Attention should be directed to the fact that it was necessary to alter the relation of the pressure curves to the base line between segments H and K in order to register the high pressures existing.

third and fourth stages (fig. 1) could only lead to the inference that the systolic discharge had increased despite the marked cardiac acceleration, but a glance at the actual pressure curves reproduced in figure 2 shows that this conclusion needs to be modified and at some points of the experiment must certainly be reversed. The great intensification of the primary oscillation, the subsequent development of after-vibrations, the marked diminution in the length of the ejection period, together with the prompt fall of pressure during systole or at the very beginning of diastole inclines to the view that the volume discharged by each beat is really reduced. The great temporary elevation of systolic pressure is due to the increased initial velocity of ejection. With such an interpretation a continued increase in peripheral resistance, or change in elasticity of vessel walls must be assumed to take place.

Does the passage of radio waves have a specific effect apart from the thermal? In some respects a careful study of the circulatory changes offers greater hope of solving this question than does a study of blood and metabolic changes referred to above. The significant differences between the circulatory changes reported by us and by Cheer (1928) naturally suggest that the passage of radio waves may have an influence apart from the thermal changes induced. In a general way, this influence seems to be such that blood pressures are elevated, not decreased, and that the force of ventricular contractions is increased rather than depressed. Such general impressions must not, however, be confused with scientific proof. We therefore sought to obtain further information on the subject by varying the experiments. In some instances, as in the one illustrated by figures 1 and 2, radiothermy was discontinued for short intervals. During these periods, the heart temperature as well as the heart rate remained unchanged. The pressure changes during two such periods of interruption are illustrated by segments D, E, F and H, I, J of figure 2, respectively, and the numerical changes in systolic and diastolic pressures are shown at corresponding letters on the chart of figure 1. It is obvious at a glance that both systolic and diastolic pressures decreased promptly after interrupting radiothermy and promptly mounted again after its resumption. These clear-cut observations do not necessarily predicate that changes aside from local thermal variations in cardiac muscle occurred. It is not difficult to understand that any form of apparatus which produces a primary intracellular rise of temperature might cause a considerable local heating of these tissues before a detectable change in temperature of the circulating blood manifests itself. Hence we were not satisfied that the absence of parallelism between heart temperatures and cardiac effects is adequate experimental proof for the belief that radiothermy exerts its effect otherwise than through local increases in temperature.

In another group of experiments the effects of radiothermy were noted

before any increase in temperature occurred. As a rule, this likewise caused an increase in pulse pressure previous to a rise of heart temperature. Frequently also, a slight retardation of the heart rate occurred (cf. segments A and B, fig. 2). These effects were not instantaneous, however, but required 2 to 3 minutes of radiation before they developed. This again supports the view that some physical or chemical change must first occur before alteration of the heart beat or vascular system is introduced.

Finally, we tested the effect of radiothermia on dogs whose temperature, owing to a cold environment, had fallen to lower levels. The effects on the circulation could thus be studied previous to the development of normal temperatures (37.5 +°C). Segments of pressure records from such an experiment are reproduced in figure 3. The first five segments (A to E) show the progressive changes taking place within 31 minutes of such radiation, during which the animal's temperature was raised from 35 to 36.5°C. Segment C, recorded 6 minutes after radiation and without recognizable change in temperature, begins to show a few elements of the characteristic changes: the diastolic pressure is higher and the diastolic gradient is less. In segments D and E these changes are more pronounced, and, in addition, the amplitude and contour of the curve and the duration of systolic ejection are significantly affected. This supports our previously expressed opinion that the initial changes which occur are vascular rather than cardiac. When heart temperatures reach normal after 74 minutes of radiation (segment F) the heart rate slows somewhat and diastolic pressure during apnea (beats 1 and 2) is slightly reduced, as is the pulse pressure. This slight decrease in heart rate is similar to that observed by Cheer (1928) in animals whose supernormal temperature was restored to normal by external heat. These experiments and others of a similar nature give no evidence that the passage of radio waves has a direct action on the heart previous to elevation of temperature, but it does appear to produce a slight vascular change, the nature of which requires further study.

The effects of prolonged hyperthermia and changes during recovery. In some of our experiments radiothermia was continued until heart temperatures reached approximately 41°C. and the animal was then allowed to cool at such a rate that a hyperthermia above 39.8°C. had existed in the animal for a period of approximately two hours. Most of the animals so studied were given small doses of morphine in order to prevent the excessively deep breathing otherwise produced, thus the reactions corresponded more nearly to those observed in man. They had the further advantages that blood changes were probably not significant and that pressure variations could still be compared during phases of apnea. The records of figure 3 were taken from such an experiment. In segment F pressure variations obtaining at a temperature of 38.9°C. are shown. We note at a glance the slowing of the pulse already referred to and in addition a respiratory

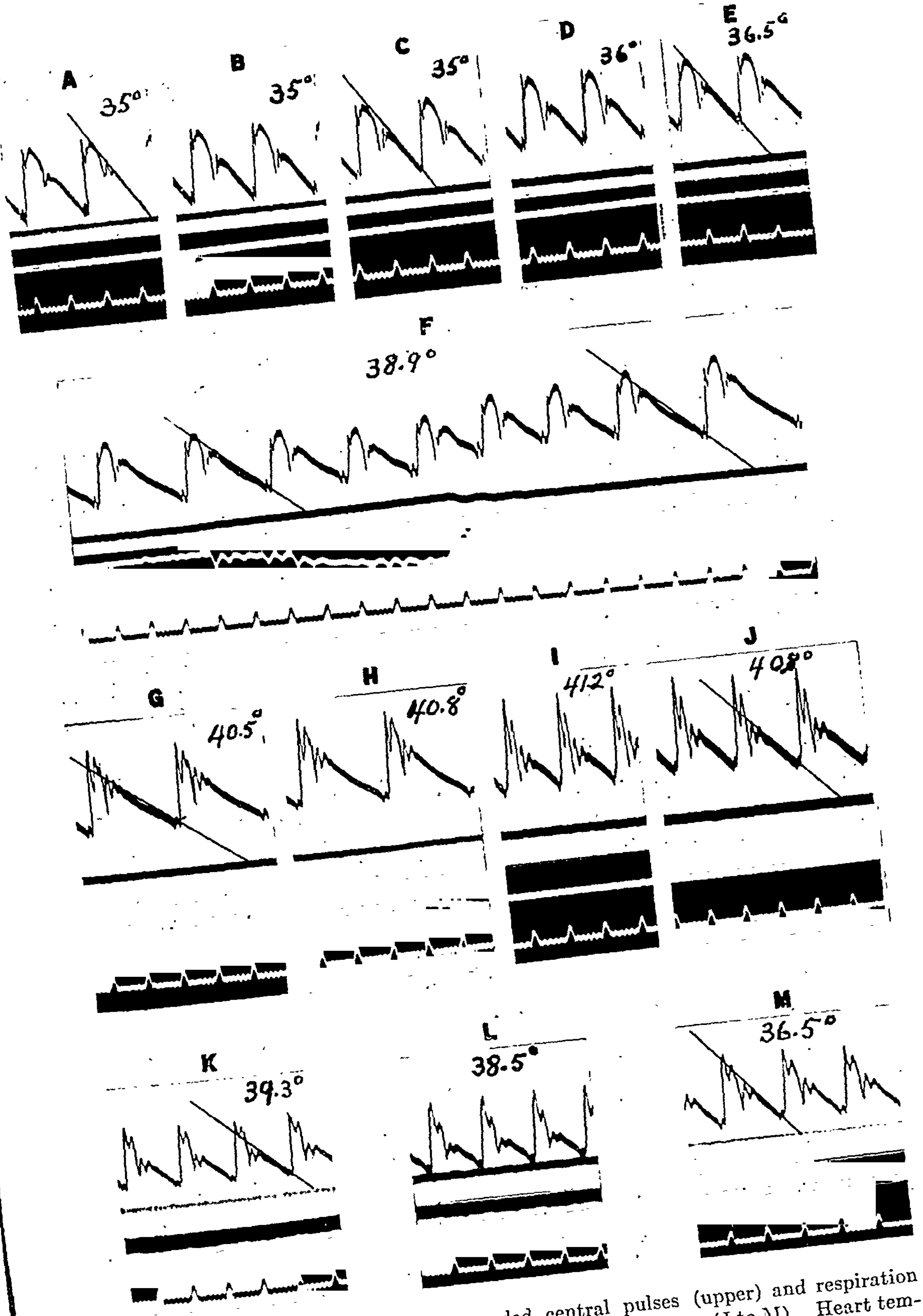

Fig. 3. Segments of optically recorded central pulses (upper) and respiration (lower) during radiothermy (A to I) and subsequent recovery (J to M). Heart temperatures inscribed directly on each segment. (Greatly reduced and slightly retouched for reproduction.) Time $\frac{1}{5}$ second. Discussion in text.

arrhythmia which, as a matter of fact, developed somewhat earlier. The entire curve is reproduced to demonstrate the variations in diastolic pressure, pulse pressure, contour of curve and duration of systolic ejection that may be expected at different cycle lengths when temperatures are still normal for the dog. Subsequent changes induced by radiothermia can be compared with it to advantage. As the temperature rose progressively at 41.2°C. (segment I) the circulatory changes followed practically the same course regardless of the fact that respiration remained very slow. Segments G, H and I illustrate the most significant alterations. They require no further discussion, except to state that at the time segment I was taken the animal's temperature had exceeded 39.7°C. for 30 minutes. Immediately after this record had been taken radiothermy was permanently discontinued and the subsequent segments show the changes during recovery. It will be noted in segment J, taken 22 minutes later but when the heart temperature was still 40.8°C., that no very essential alterations in the heart's action had taken place. Although the heart cycle is exactly the same as that of segment C the systolic ejection is only 0.071 second as compared to 0.12 second in the beats of segment C. However, the diastolic pressure especially is lower and the diastolic gradient less steep. As cooling continues to 39.3°C. (segment K) and to 38.5°C. (segment L) both systolic and diastolic pressures fall considerably and pulse pressure is greatly reduced. It is quite obvious that the systolic discharge is seriously impaired and that ventricular ejection does not occur with normal vigor. Segment M shows the effects two hours after discontinuing radiothermy and 45 minutes after taking record L. The temperature had fallen to 36.5°C. The curves show that some degree of recovery occurred. Compare these beats with those of segment A, however. We note very similar systolic and diastolic pressures, but the contour of the curves clearly shows the existence of a hypodynamic state of the heart and circulation. Calculations show that systolic ejection is still very short, that the heart remains accelerated and that volume of systolic discharge is still below normal.

We must therefore conclude that prolonged radiothermia even when temperatures do not reach precritical levels is followed by severe circulatory reactions during the process of cooling and after the restoration of normal temperatures. While irreversible dangerous hypodynamic states from which recovery is impossible (circulatory failure) have not been encountered in our experience the reactions are sufficiently severe to suggest the need of greatest care and caution in the handling of human subjects immediately after exposure to the radiotherm.

SUMMARY

The circulatory reactions during radiothermia (i.e., hyperthermia produced by passing short radio waves through the body) were investigated

in anesthetized dogs by registering the pressure pulses from an innominate artery by a mirror manometer.

Several types of experiments were carried out: 1. In some heating by radio waves was continued until death. 2. In others radiothermy was interrupted for varying periods and at different temperature levels. 3. In still others, hyperthermia was maintained between 39.8 to 41° for an hour or two, and the recovery studied.

Qualitative and quantitative studies of the circulatory reactions reveal certain differences in hyperthermias produced by high external temperatures and by radiothermy. In hyperpyrexia due to the former, blood pressures tend to decline with temperatures above 41°C. The pressure pulses become smaller, rise more gradually to a peak and are devoid of all secondary vibrations. In short, they display features which suggest that systolic discharge is reduced and the force of ventricular contraction decreased. During radiothermia, on the other hand, systolic and diastolic pressures increase tremendously after heart temperature exceeds 41°C. The pressure pulse increases greatly in amplitude, rises sharply to a peak and contains many after-vibrations. All of these features suggest that the force and vigor of ventricular contraction are improved until the crisis is reached. Many features of the curves suggest, however, that the systolic discharge delivered by each beat may not be increased and perhaps is decreased. Vascular changes doubtless contribute to the blood pressure changes, but whether these are in the nature of an increase in peripheral resistance or a decrease in elasticity of vessel walls requires further study.

Despite the fact that changes in heart rate, pulse pressure and pulse form have been observed without changes of temperature within the heart cavities, this does not prove that the passage of radio waves exerts an effect independent of local temperature changes in the cardiac or vascular structures.

When radiothermy is continued long enough, death occurs as in other forms of hyperthermia, i.e., through primary failure of the respiratory center which is followed by circulatory failure due to asphyxia.

Animals kept between temperatures of 39.8 to 41° for two hours manifest a circulatory reaction as the temperature declines and returns to normal. The blood pressures fall and pressure pulses clearly indicate that systolic discharge is decreased and ventricular expulsion is less forceful. These reactions combined with vascular relaxation may cause hypodynamic states which, though not to be classed as circulatory failure, are nevertheless severe reactions. Hence the suggestion is made that great care and caution are advisable in the aftercare of patients exposed to prolonged radiothermy.

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THE DYNAMIC CHANGES IN THE VENTRICLES FOLLOWING LIGATION OF THE RAMUS DESCENDENS ANTERIOR

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Although the disturbances of cardiac rhythm and the electrocardiographic changes subsequent to ligation of large coronary branches have been repeatedly investigated in recent years, the dynamic changes which follow when irregular action is transient or absent have not been extensively investigated. Indeed, the studies of W. T. Porter (1894, 1896) appear to be the only ones on record in which dynamic changes in the heart and circulation were more than incidentally investigated. Porter recorded left ventricular pressures by a Gad manometer and succeeded in ligating the ramus descendens anterior without the accompanying veins. His results showed clearly 1, a gradual and continuous decrease in the height of ventricular pressure curves; 2, a slower gradient of upstroke and of diastolic downstroke, and 3, an increase in initial tension preceding cardiac standstill (fibrillation). More recently, Sutton and Lueth (1930) as a minor part of their program for investigating the cause of cardiac pain reported that ligature of large cardiac branches causes an immediate fall of mean arterial pressure which is prevented however by section of the vagi nerves. No explanation is given for this remarkable effect. Feil, Katz, Moore and Scott (1931) who also recorded mean arterial pressures found more instances in which no alteration of pressure or even a rise occurred than cases in which it fell, regardless of the fact that the vagi were left intact. Sutton and Lueth (1930) also registered the contractions of the right auricle and ventricle by means of a myograph. The published tracings seem to show an increased amplitude of right ventricular contractions and a considerable dilatation during diastole. The methods employed are scarcely adequate, however, to establish these facts with surety (cf. Wiggers, 1923).

If the results of Porter and those of Sutton and Lueth are given equal value we are left with the conclusion that the left ventricular contraction diminishes while that of the right increases. The validity of such con-

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clusions, as well as the reasons for such effects, however, require further check and study by more precise methods.

METHODS. Large dogs were chosen and anesthetized with small doses of morphine and barbital. The chest was opened under artificial respiration. Pressures were recorded from the left and right ventricles, aorta and pulmonary arteries, two at a time and in various combinations by Wiggers universal optical manometers. The usual technique discussed by Wiggers (1928) was followed. Electrocardiograms were always recorded (usually lead II) and in some experiments mean carotid pressure and kidney volumes were recorded in addition.

For the sake of uniformity the *ramus descendens anterior* was ligated 1–2 cm. from its origin. The vessel was dissected free from accompanying veins and tissue, and the isolated vessel was ligated. As a rule, but not always, the ligature was applied above the origin of the first branch supplying the conus region of the right ventricle.

Continuous optical records were taken immediately before and after drawing tight the ligature so that the consecutive effects for the first 30 seconds were recorded. After this, records were taken at 1 minute intervals for 4 to 6 minutes, then at longer intervals until death from fibrillation occurred or up to one hour in case of survival.

In order to avoid changes in heart rate—so essential in evaluating cardiodynamic changes—as well as to create a slower tempo, the S-A node was clamped in approximately half the experiments. The vagi nerves were always left intact.

General course of experiments. Earlier investigators found that ventricular fibrillation terminates the experiments relatively soon after ligation of the ramus descendens anterior. Thus, in Porter's experiments arrhythmia and tachycardia followed by fibrillation occurred 65 to 264 seconds after ligation; subsequent experimenters (Miller and Matthews (1909), Karsner and Dwyer (1916), Smith (1918)) demonstrated that fibrillation is not a necessary accompaniment of coronary vessel ligation and that the mortality can be reduced to about 9 per cent, the remaining animals living for many months.

In my experiments fibrillation occurred more frequently. This may be due to several factors; among them, that the vessel was ligated nearer its origin and that nonvolatile anesthetics and more complicated experimental procedures were required in my dynamic studies. For example, one ventricular manometer was always inserted through the ventricular wall and in case of left ventricular pressure registration this was inserted through the area supplied by the *ramus descendens*.

Nevertheless, 23 good experiments were realized as far as information on the dynamics of the ventricles was concerned. In 11 dogs fibrillation occurred 4 to 26 minutes after ligation without signs of a hypodynamic

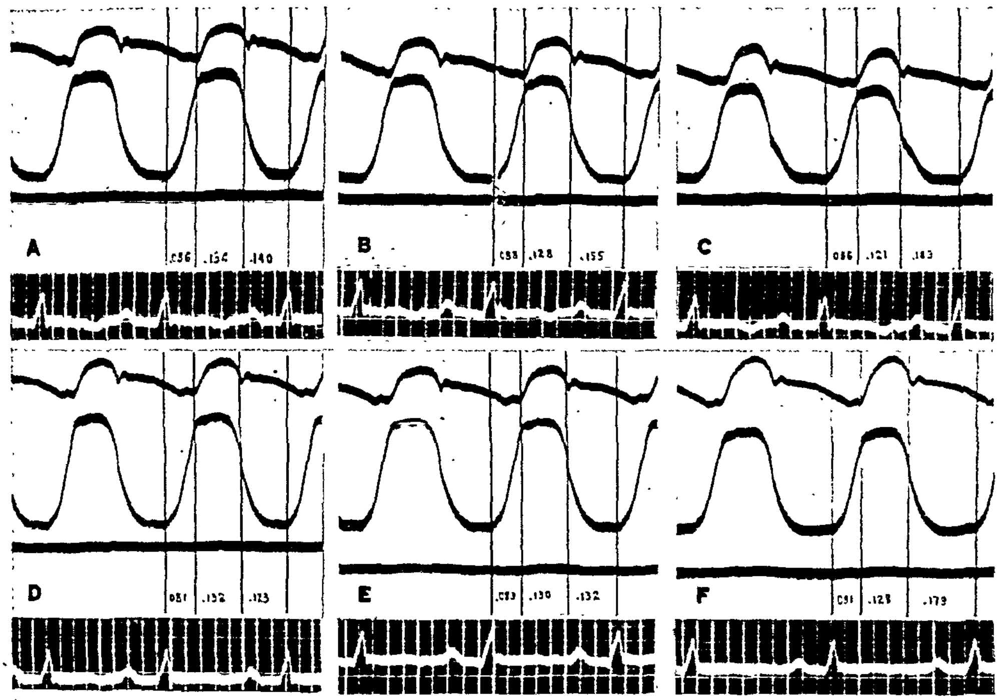

FIG. 1

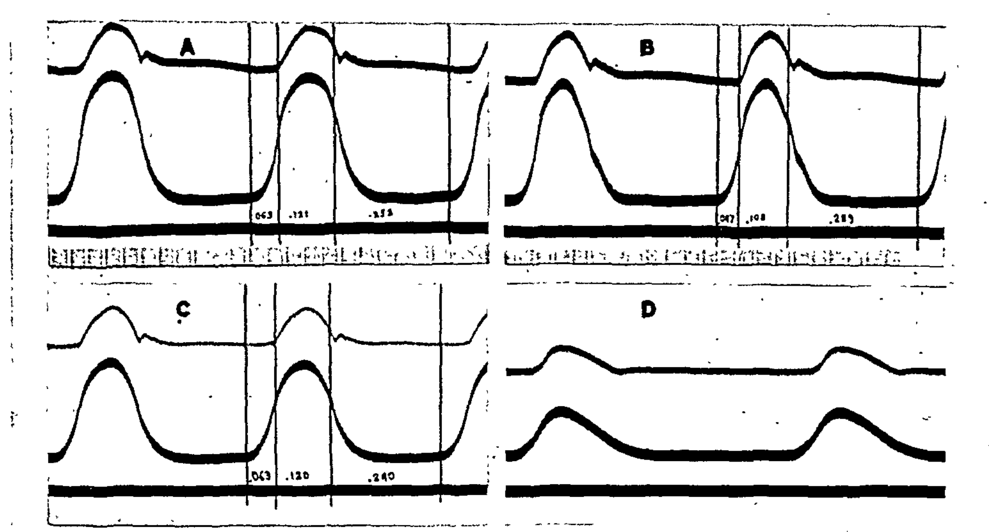

FIG. 2

Figs. 1 and 2. Aortic (upper curve) and left ventricular (middle curve) pressure curves with E.C.G. lead II. Showing effects before and after ligating ramus descendens anterior. Discussion in text.

condition preceding fibrillation. In two experiments fibrillation followed premature contractions produced by accidental mechanical stimulation. Seven dogs survived for an hour or more and the experiments were ter-

minated voluntarily. Three dogs displayed a progressive failure and after the heart had become very weak died in 15 to 25 minutes in final fibrillation.

Description of records. The dynamic changes following ligation at different intervals up to 1 hour in a favorable experiment are illustrated in figure 1. Segment A illustrates curves obtained before tying and segments B and C the effects during the 17 beats following ligation. The most obvious and immediate changes in the aortic pressure curve (upper) are the decrease in systolic and diastolic pressures and the changes in its contour, the rise being more gradual and the summit more rounded.

The left ventricular pressure curves (middle curves) rise more gradually and reach a lower and more rounded crest. The initial pressure is slightly elevated. In short, the chief findings of Porter are substantiated by use of more efficient manometers. Calculations show, in addition, that the duration of contraction is decreased very significantly and that the change occurs without alteration of the isometric phase. This occurred despite a slight increase in the duration of the entire heart cycle (0.36 $<$ 0.37 sec.). Reactions of this type were obtained in more than half of the experiments.

The conclusion is reached that ligation of a large coronary artery may be immediately followed by a hypodynamic beat characterized by decreased amplitude and abbreviation in contraction. The changes in blood pressure are obviously a consequence of the reduced systolic discharge, and could not possibly be affected by vagus section.

As shown in segments D, E and F, which were recorded 4, 9 and 60 minutes respectively after complete ligation, such hypodynamic effects do not persist long. In as short a time as 4 minutes after ligation (segment D), aortic and ventricular pressures are not only restored to normal but actually indicate that the heart action is dynamically somewhat better than normal. The systolic pressure maxima are higher, the form of the curve is normal again and the period of contraction lengthens again. The chief alterations are a definite rise of initial tension and an increased diastolic size of the ventricle, noted on inspection. The longest interval noted for such complete recovery was 7 minutes. Segments E and F show that such dynamic recovery persists for a long time; but the initial tension continues to rise and the duration of contraction tends to decrease slightly again.

The conclusion is clear that compensatory mechanisms very promptly come into play to restore ventricular action and blood pressures to normal provided the heart is not hampered dynamically by too many unfavorable premature contractions and provided, of course, that it escapes early fibrillation.

In three of the 23 experiments the compensatory changes of the left ventricle noted above failed to occur. The heart dilated progressively and the beats became visibly weaker. The course of the pressure changes are exemplified by records reproduced as figure 2. The aortic pressures decline

progressively while the ventricular pressure maximum becomes progressively lower despite the progressive increase in initial tension. However, comparison of segments A and C show that the initial abbreviation of contraction shown in segment B does not persist. The curves of segment D illustrate the failure of the left ventricle 3 minutes before eventual fibrillation supervened.

These exceptional experiments are reported because they probably give an idea of the mechanisms through which the heart eventually fails in animals and men that survive for comparatively long intervals of time.

The records of figure 3 illustrate another type of reaction which occurred in approximately one-third of my series, viz., a condition in which the systolic pressure maxima in aorta and left ventricle were not primarily decreased, but on the contrary, increased. In these animals the pronounced abbreviation of systole is the only characteristic, the cardiac cycle remaining practically unchanged (cf. segments A and B). Within 4 to 6 minutes later systolic ejection lengthened again as shown in segment C and the configuration of the pressure curves was restored to normal. Enlargement and superposition of the left ventricular pressure curves of segments A and C show evidence of a slight though unmistakable increase of initial tension not easily seen in records reproduced in reduced size. Observation revealed a considerable increase in the diastolic size of the ventricles at the time.

The records of figure 3 also illustrate one type of right ventricular response, viz., a slight decrease in the contraction amplitude of the right ventricle in segment B while that of the left increases. The duration of right ventricular contraction is also diminished but owing to the indefinite character of the incisura exact calculations are difficult to make. For this purpose, studies of simultaneous aortic and pulmonary arterial pressure curves are really better. Several experiments in which such records were obtained were carried out. In each it was found that the decrease in the contraction period of the right ventricle was much less than in the left and recovery occurred earlier. The following brief tabulation of results from experiment AA-42 illustrate the changes when the cycle remained unaltered.

	EJECTION PHASE, LEFT VENTRICLE	EJECTION PHASE, RIGHT VENTRICLE
	seconds	*seconds*
Normal	0.137	0.160
11 seconds after ligation	0.096	0.140
20 seconds after ligation	0.087	0.147
5 minutes after ligation	0.125	0.152

In other experiments however the right ventricular contractions showed no obvious changes while the left was characteristically depressed. An example of this type of response is reproduced in figure 4.

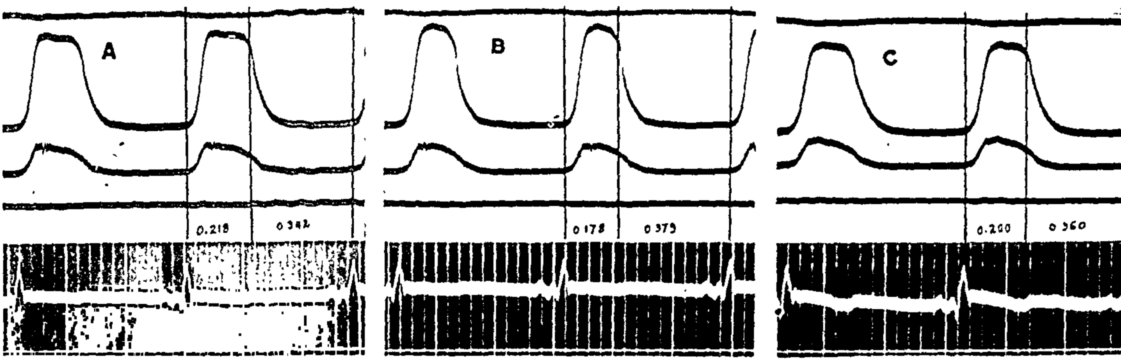

FIG. 3

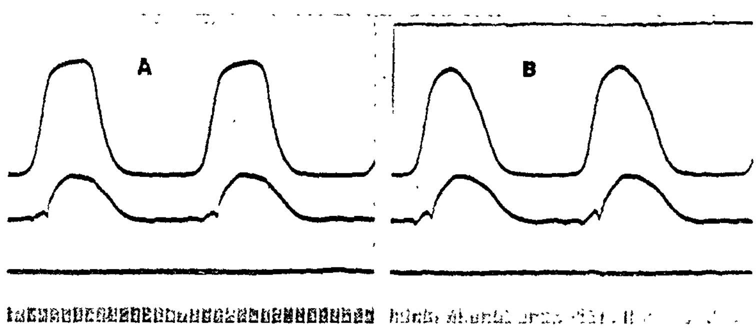

FIG. 4

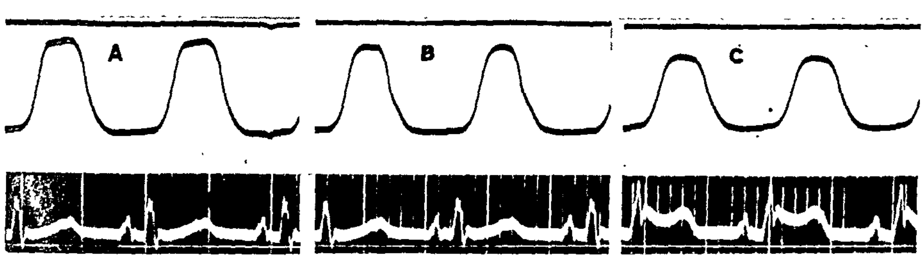

FIG. 5

Figs. 3 and 4. Left (upper curve) and right (middle curve) ventricular pressure curves with E.C.G. showing effects before and after ligating ramus descendens anterior. Discussion in text.

Fig. 5. Left ventricular pressure curve and E.C.G. (lead II) showing simultaneous changes in configuration. Discussion in text.

It is obvious that the changes in the dynamics of the two ventricles may be dissimilar or again that the alteration in beats may be limited to the left ventricle. In no instance was an increased vigor of right ventricular contraction found, however.

The relations found between electrocardiographic changes and dynamic alterations particularly in the left ventricle may be briefly referred to. Consonant with observations of others no early changes in the electrocardiogram were found as a rule. Apparently the characteristic changes in the electrocardiogram deflections are either delayed (Smith, 1918) or the result of additional ischemia of the heart muscle (Feil, Katz, Moore and Scott, 1931). The complete absence of significant alterations is illustrated in the electrocardiogram of figure 1.

In figure 3 however the electrocardiograms indicate a tendency to form a negative T wave and this despite the fact that the dynamic efficiency illustrated by pressure curves is essentially normal. Figure 5 is added to illustrate one of three cases in which a characteristic monophasic ventricular complex developed rather quickly after ligation. Its early appearance in association with a greatly dilated and hypodynamic heart (evident from pressure curves) would be significant were it not for the additional facts: that 1, in the two other cases in which the heart failed progressively, the electrocardiogram remained perfectly normal, and 2, during a recent class demonstration a similar alteration of the electrocardiogram occurred when the coronaries had not been ligated and no cardiodynamic disturbances coexisted. My experience leans toward the conclusion that no relationship exists between changes in the electrocardiogram and the dynamic changes in the ventricle; either may occur without the other and their coexistence may be entirely a matter of chance.

Discussion. The initial and almost immediate changes in the dynamics of the left ventricle after coronary ligation fall into two general classes: a, those in which a preliminary phase of slight hypodynamic action occurs, to be followed by a phase of quick recovery except for a continued and pronounced abbreviation of contraction, and b, those in which a prompt reduction in the duration of the contraction phase is the only abnormality. Careful study of the results inclines to the belief that the two types of reactions are fundamentally identical in the sense that in some hearts the two processes occur together and in others follow in succession.

Two views as to the nature of these dynamic changes are possible: 1, that an alteration of contraction occurs in all myocardial elements of the left ventricle, and 2, that functional changes are limited to muscle tissue in the area whose blood supply is interrupted. Since crucial evidence on these alternative explanations cannot be adduced, the writer wishes to maintain an open mind toward either possibility but at present regards the latter as the more logical assumption. Anatomical preparations of the dog's heart made by Moore and described by him (1930) have shown that a considerable area of the left ventricle, approximately the anterior third of the thick muscular septum and a smaller portion of the right ventricle adjacent to the coronary sulcus is acutely deprived of its chief source of

blood supply by my method of ligation. In consequence, a local tissue anoxemia supervenes despite any existing collateral circulation with other coronary branches or the Thebesian system. This is evidenced by the cyanotic rather than pale color of the middle region of the anterior surface of the heart shortly after ligation of the ramus descendens. Unless evidence can be adduced to the contrary, it must be assumed that changes in these impoverished areas of muscle tissue rather than less probable changes in the remainder of the heart muscle determine the dynamic reaction of the ventricle as a whole. Furthermore, it is impossible at this time to picture any mechanism which acting on the entire ventricular myocardium can produce such a prompt and significant abbreviation of systolic ejection. Sands and De Graff (1925) found that anoxic anoxemia has such an effect; but there is no reason to suppose that other regions of the heart suffer an oxygen lack after ligation. Feil and Katz (1923) noted a decrease in duration of systole in decompensated hearts which they attribute to overdistention; but at this stage the hearts of my animals were not significantly distended and recorded pressure curves failed to show any appreciable rise of initial tension. The hearts certainly were not decompensated in any sense. Accelerator nerve stimulation (Wiggers and Katz, 1920) and epinephrin (Wiggers, 1927a) produce a similar abbreviation of systole; but the hearts of animals after coronary ligation showed neither acceleration nor amplitude changes consistent with the assumption that adrenalin-like substances are produced in the anemic areas and distributed to other regions of the heart.

On the other hand careful study has shown that the changes noted are all capable of explanation by local changes on the hypothesis advanced by Wiggers (1927) that ventricular pressure changes are the resultant of successive summated fractionate contractions. In figure 6 is shown such a resultant curve A derived mathematically from summation of a series of six hypothetical fractionate contraction curves drawn below. If however only one of these curves—indicated by dotted line—be deleted and the remainder summated, the curve B is derived. Its characteristics are a lower pressure maximum and a marked reduction of systole which, in an afterloaded manner of contraction, would show itself by a great reduction of systolic ejection (x-y). This is identical with the first and predominant change found in the majority of my animal experiments. We need only suppose that muscle fractions in the anemic area respond with less vigorous contractions to realize the result expressed graphically by complete deletion of one-sixth of the fractionate contractions. Hence the deletion of a fractionate portion of the contractile force in the anemic area is offered as an explanation of the diminished vigor and decreased duration of the contraction found in a preponderance of experiments.

But the response of cardiac muscle apparently changes when anoxemia

reaches a certain degree,—the velocity of tension development increases and the duration of contraction shortens. This was demonstrated by Sands and DeGraff (1925) and confirmed by the studies of Strughold (1930) by testing the response of the whole heart to anoxic anoxemia.

If, as illustrated in figure 7, a series of six successive fractionate contractions, the 4th of which rises more steeply and terminates earlier, be added algebraically the resultant curve C is obtained. We note that it has practically the same height as curve A but the duration of systole and, in an afterloaded mode of contraction, the ejection phase (x-y) are also reduced. This is identical with the reaction observed in a second group of experiments exemplified by curves in figure 3. Hence the suggestion that in-

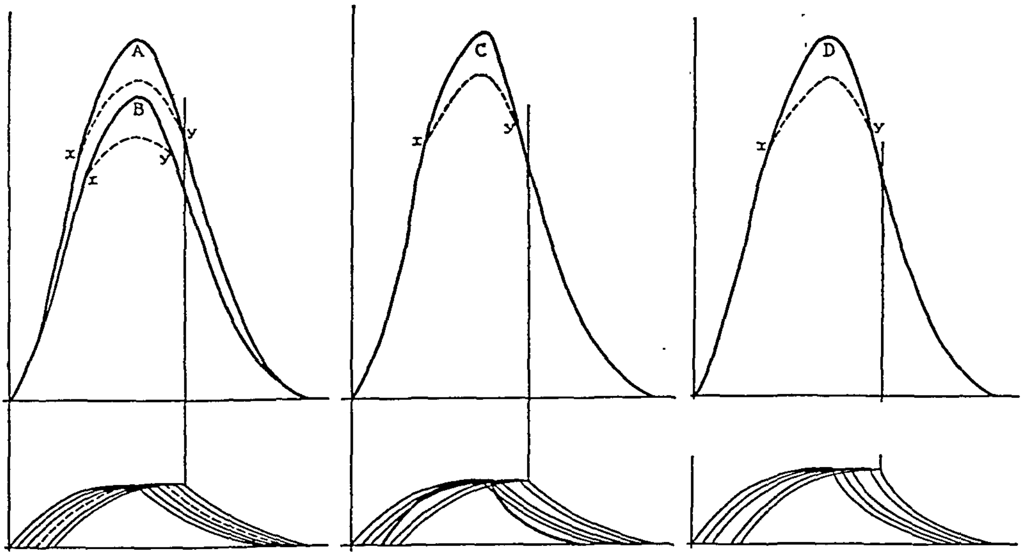

Fig. 6 Fig. 7 Fig. 8

Figs. 6, 7 and 8. Constructions showing theoretical variations in contour and temporal relations of ventricular pressure curve (upper curves) produced by variations in number and contour of fractionate contractions (lower curves). Discussion in text.

creased velocity of contraction combined with a shorter duration of contraction in the muscle units of potentially infarcted areas is the cause of those dynamic reactions which show abbreviation of systolic ejection alone.

It is not the contention that one of these two types of reactions occurs to the exclusion of the other in any given heart after ligating the ramus descendens branch of the anterior coronary artery, but rather that both effects occur side by side in the potentially infarcted area, with one effect dominating. The dominance is easily explained when the variable degree of collateral circulation found in different hearts of dogs and the many

physiological factors which determine their functional efficiency are taken into account. It is quite possible by summating large numbers of fractionate contractions to produce the same effect as shown in curve B by deleting one but intensifying and shortening another fractionate contraction curve. As such graphs become too complicated for successful illustration, I have resorted to the simpler graphic method for expressing my ideas.

It remains to explain the mechanism whereby the duration of ventricular systole gradually increases and the contour as well as the ultimate pressure maximum are restored to normal even though vessels remain occluded.

The simplest explanation would be that as a result of a continued improvement in collateral circulations a gradual recovery of the normal contraction mechanisms occurs in the potentially infarcted area with the result that the conditions depicted in curve A of figure 6 are restored. Two facts are opposed to such a conclusion. It has been demonstrated conclusively that hemorrhagic infarcts which later undergo decolorization and cicatrization occur in dogs after ligating the ramus descendens provided animals are allowed to survive (Karsner and Dwyer, 1916). Since this clearly indicates that the collateral circulation is not adequate to maintain nutrition in later stages it is also unlikely that it becomes sufficient to revive functionally damaged muscle during the period of these experiments.

Furthermore, it has been a constant observation that at the time of such recovery the ventricle is definitely dilated. Pressure curves from the left ventricle reveal a measurable increase in the initial tension. In other words, the contraction pattern of the potentially infarcted heart is restored to normal only after initial tension and increased diastolic size have increased, consequently, the obvious suggestion that the increase in *diastolic size is responsible for the recovery and that it acts by affecting* chiefly at least the undamaged muscle of the ventricle. The effects of such a mechanical stretching are well known; it increases the duration as well as the amplitude of contraction in the normal heart and according to the experimental evidence of Wiggers (1927) does so exclusively by a similar action on the component fractionate contractions. The effects of such an increase in the magnitude and duration of fractionate contractions when one of a series of six is eliminated is shown in curve D of figure 8 which is the algebraic sum of the curves shown below. Comparing curves A and D in figures 6 and 8 we note recovery to nearly a normal amplitude and form as well as reinstatement of normal contraction and systolic ejection phases. Coupled with an increased contraction velocity of some of the fractions as illustrated in graph C of figure 7 it could actually lead to a higher pressure maximum and normal values of systolic ejection such as are illustrated in the optical records of figure 1, D, E, F. Thus it may

quickly come about that the dynamic efficiency of the ventricle as a whole is improved, though at the expense of a decreased reserve power.

The ability of the ventricles to compensate in this manner depends largely on the condition of the unaffected heart muscle; if it is unable to respond in the characteristic physiological manner, the heart dilates more and more and eventually responds by much feebler contractions as illustrated in figure 2. As is well shown in these curves, failure to respond by larger contractions to increased initial tension does not necessarily prevent the subsequent prolongation of contraction, a divergence of phenomena which Doctor Wiggers informs me has been noted in other circumstances. The separate factors concerned obviously require more detailed investigation.

After ligation of the ramus descendens, the dynamics of the right ventricle was found either to be unaltered or to show changes similar to but much less in degree than those in the left.

While my time limitations in the laboratory made it impossible to check all the variables that may enter and particularly changes in venous return, a careful study of individual experiments and of the gross distribution of the coronaries below the ligature suggests that the dynamics of the right ventricle remain unaffected when the first rather constant branch supplying the conus region of the right ventricle was missed by the ligature, but a moderate decrease in amplitude and duration of systolic ejection took place when the descending ramus was tied above its origin. This interpretation proved more convincing after a careful restudy of Doctor Moore's preparations and x-rays of the injected coronaries, kindly placed at my disposal by Professor Karsner. That a considerable region of the conus and basal region of the right ventricle may be supplied by this branch cannot be doubted.

Hence the suggestion is made that when an adequate region is deprived of its supply from the anterior coronary descendens branch the reactions of the right ventricle can be explained as are those of the left. Owing to better collateral circulation, recovery occurs more rapidly however and in this case may indeed be concerned with recovery.

SUMMARY

The changes in ventricular contraction following ligation of the isolated ramus descendens branch of the anterior coronary artery in the dog were studied by recording pressure pulses from the ventricular cavities, aorta and pulmonary artery by means of optically recording manometers.

In the preponderance of experiments, ligation was followed immediately by hypodynamic beats characterized by decreased pressure amplitudes and a significant reduction in the intervals of systole and systolic discharge. In consequence of the reduced systolic output, systolic and diastolic pressures fell. These effects however were promptly compensated for (4–7

min.) through an increase in diastolic size and a rise of initial tension which restored systolic pressures in the left ventricle and aorta as well as the duration of contractions to normal. Occasionally a slight overcompensation occurred so that the ventricle action as a whole was dynamically supernormal.

When the condition of the heart muscle is so poor that its power of responding properly to such changes in initial tension is lost, the ventricles progressively dilate, the beats become more feeble, the pressure in ventricles and aorta falls and acute myocardial failure supervenes.

In approximately one-third of the experiments, systolic pressures in the aorta and ventricle were not decreased primarily, but tended to increase. In these hearts the typical initial abbreviation of contraction was always present however.

The dynamics of the right ventricle and pulmonary circulation were either unaffected or showed changes similar to though less in degree than those described as characteristic for the left side of the circulation. The differences are attributed to the extent to which potential infarction of the right ventricular muscle occurred in different ligations.

Electrocardiographic changes were incidentally recorded. As a rule, no significant changes in the character of deflections occurred; more rarely, a tendency to development of negative T or monophasic initial deflections was noted. Both occurred without regard to the dynamic conditions of the ventricles as judged by ventricular pressure curves.

Two views as to the cause of the changes in ventricular action after coronary ligation are discussed, viz., 1, that the contractile force and duration are changed in all muscle units of the ventricle involved, and 2, that the changes observed are due to modifications or deletion of fractionate contractions in the potentially infarcted area. In brief, the conclusions are reached: 1, that the primary depression of contraction and decrease in ventricular systole are caused by deletion of fractionate contractions in potentially infarcted areas; 2, that absence of the initial depression may be accounted for by primary anoxemia of some affected muscle fractions, but 3, that the real compensation by which duration of contraction and normal pressures are restored and maintained is due to the increase in diastolic size and initial tension. Through the response chiefly, if not solely, of unaffected muscle the contractions of the ventricle are restored to normal. Attention is called to the fact, however, that this occurs at the expense of the reserve power of the ventricle affected.

In conclusion, I wish to express my gratitude to Prof. Carl J. Wiggers for his guidance and help and also for the facilities offered in his department.

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THE LYMPH CAPILLARIES IN THE WEB OF THE FROG

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Direct observation of blood capillaries has become an easy and useful method for study of problems of transudation and absorption. This ease of observation is due to the contained red cells. It is the absence of a visible content which has made direct observation of most lymphatics so difficult.

Even in fixed and stained preparations the lymph capillaries are usually poorly defined as compared with the blood capillaries, and many points of prime physiological importance cannot be learned from even the best material. The web of the frog is one of the few capillary areas which can be examined without mutilation or disturbance. In addition to a plentiful blood supply it possesses an extensive network of lymph capillaries. von Recklinghausen (1862) described the injection of these lymphatics, and published a very characteristic picture of them. He employed a Berlin blue mass and injected by means of a needle thrust into the subcutaneous lymph sac near the ankle joint. He reported that much pressure was necessary in order to cause the mass to enter the lymphatic network, but when the flow started it spread abruptly. von Recklinghausen found no evidence of extravasation, and concluded these vessels were not continuous with the tissue spaces—a conception out of accord with his principle of direct communication. Langer (1867) duplicated von Recklinghausen's injections and provided a drawing of the lymphatic net together with the blood capillaries. One of the physiological issues which has interested us is the comparative absorbing surface offered by these two systems in a known area of tissue. Unfortunately Langer's illustration, in addition to a somewhat imaginative quality, fails to give the degree of magnification. We have found no other histological examinations of the web lymphatics, and consequently have been compelled to make preparations of a number of different types in order to get all the facts possible.

Methods. All lymphatic and vascular injections have been made in the living frog, anesthetized with urethane (0.02 cc. 25 per cent urethane per gram). This results in a varying degree of capillary dilatation, but frogs recover readily from urethane, a desideratum not realized regularly with other non-volatile anesthetics (sodium veronal, nembutal).

In order to show details of lymph capillary structure we injected 2 per cent silver nitrate in water and then exposed the foot to strong ultra-violet light for 30 minutes.

To show the extent of the lymph capillary plexus the graphite mass described by Drinker and Churchill (1927) was used. This behaved beautifully if it was unnecessary to keep the capillaries filled for more than a few minutes. The mass begins to run out of the lymphatics into the subcutaneous lymph space as soon as the injecting pressure is removed, and while this complete failure to stick is often of value, it prevents keeping the same vessel in view for a long time. Injections of commercial India ink left the lymphatics more slowly, but invariably ran out leaving the vessels just as invisible as in the normal web. A convenient mass for vital injection, and one certainly fairly normal for the living animal, was made as follows. To 4 cc. of a thick ammonia free graphite suspension in 3 per cent gum acacia add 4 cc. of fresh oxalated mammalian blood plasma (0.5 cc. 1 per cent sodium chloride to 4 cc. whole blood). Determine the coagulation time of the graphite-plasma mixture on adding 1 per cent calcium chloride in 0.9 per cent sodium chloride. Select an amount of the calcium chloride solution which will give firm coagulation in about 3 minutes. Avoid shaking while obtaining this coagulation time. With all in readiness add the necessary amount of calcium chloride solution to the graphite-plasma mixture, stir, pour into a syringe and inject at once into the subcutaneous lymph sac toward the toes, inserting the needle below a ligature tied around the ankle. Another convenient method of injection is by means of a cannula tied under the skin at the tip of the middle toe. The ankle ligature enables one to raise pressure in the lymph sac by preventing escape of the injection mass up the leg. The web should be watched and as the pressure increases the lymphatics will be seen to fill suddenly. The mass will clot in 3 minutes and when solid the ligature should be removed from the ankle. After some hours the clot begins to contract and leaves the walls of the lymphatics, but enough remains to identify the position of the individual vessels.

If injection of the blood capillaries accompanies that of the lymphatics it is well to use a pump delivering an acacia-graphite mass following perfusion of the capillaries with an acacia serum perfusate. Our technique in this matter—in observation and photography—followed that described by Drinker (1927).

Observations. When one first sees the vitally injected web lymphatics it is difficult to believe the mass has not simply dissected its way through paths of least resistance in the loose alveolar tissue between the upper and lower layers of web epithelium. But it is easy to prove that one deals with a highly complicated plexus of closed tubes. Figure 1 shows that if the non-sticking acacia-graphite mass is injected and a field, A, photo-

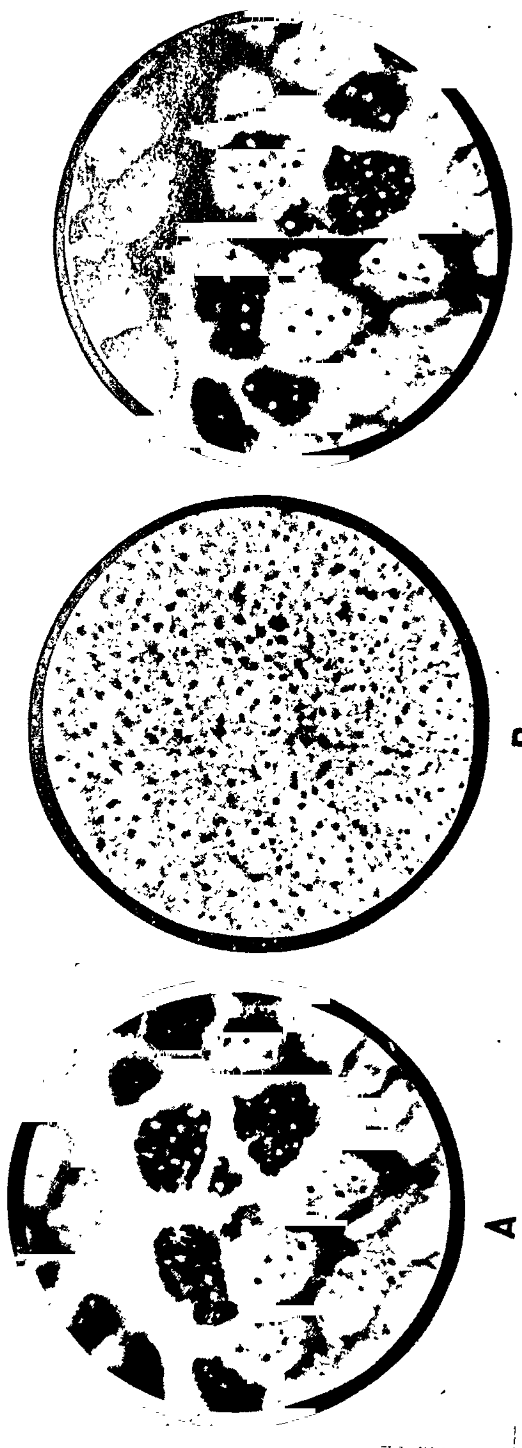

Fig. 1. Acacia-graphite injection of web lymphatics. Microphotograph A taken at 11:05, shows fully injected lymph capillaries. B shows the web at 11:11, the mass having been allowed to run out. C, taken at 11:15 after a second injection, displays the same vessel pattern as A. $\times 23\frac{1}{3}$.

graphed, the mass will entirely leave the web as shown in B and then the same vascular pattern appears when a second injection, C, is made. If the injection pressure is extremely high the mass is forced out of vessels and islets of graphite are left when the pressure is removed and the mass permitted to run out.

The entire absence of injection mass caught in the tissues is evident in B and in C there is an exact reproduction of the lymphatic network originally injected in A. These findings are inconsistent with any group of pathways save those offered by a system of closed tubes.

The lymph capillaries lie in the center of the alveolar tissue of the web. Above and below them are the two groups of blood vessels. In figure 2, a cross-section of the web cut parallel to and 0.5 mm. from the free edge, 3 lymphatic capillaries, *L*, *L*, *L*, are seen. Blood capillaries are found on both sides and usually close to the lymphatics. The lymphatics in this section were filled to distention by a graphite-plasma injection of which strands of fibrin with graphite sticking to them remain after fixation, section and staining. When high powers are used it is easy to make out an endothelial wall in these lymphatics, and with exceptional good fortune this can be traced unbrokenly around the vessel. The close

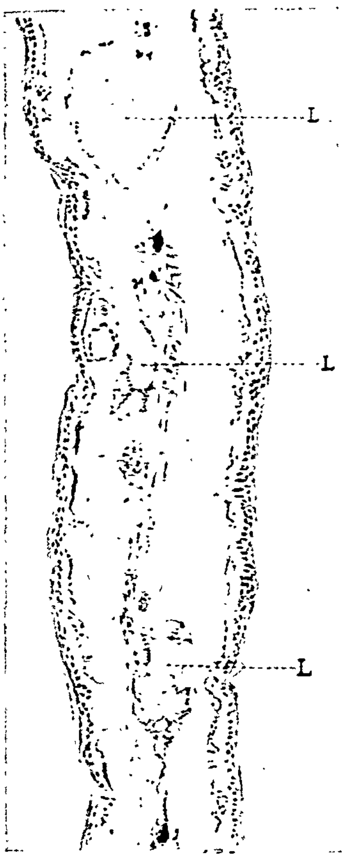

Fig. 2

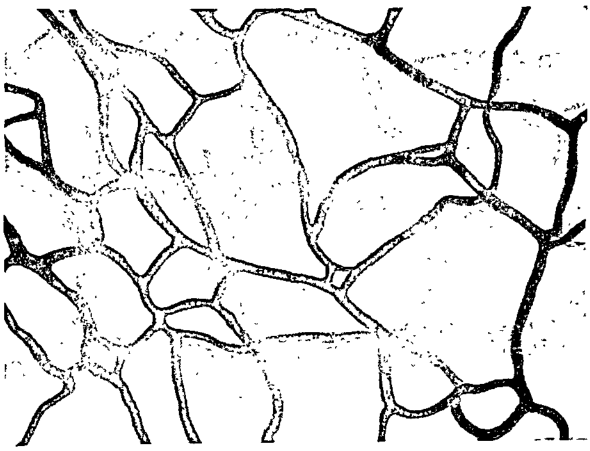

Fig. 3

Fig. 2. Camera lucida drawing section frog web 0.5 mm. from and parallel to free edge. Lymphatics *L,L,L*. Black masses immediately under the epithelium are melanophores. × 100.

Fig. 3. Tracing made from photomicrograph of the web about 0.5 mm. from free margin. Lymphatics gray, dorsal capillary plexus black, plantar capillary plexus omitted. Rectangle equals 0.74 sq. mm. of web. × 45.

relation of blood and lymph capillaries cannot be appreciated without viewing a normally circulated web in which the lymphatics are filled with an adherent plasma-graphite injection mass. The two groups of vessels are in direct contact at many points. If the lymphatics are overfilled with injection mass the circulation in the blood capillaries may be arrested and similarly if after injection of the lymphatics the blood vessels are thoroughly filled, but below the point of causing ruptures, much of the lymphatic mass may be pressed out.

Figure 3 is a tracing made from a photomicrograph of the injected lymph capillaries and the dorsal set of blood capillaries. The lymphatics are not abnormally distended. The area covered by the rectangle equals 0.745 square millimeter of the web, and is taken from a point about 0.75 mm. from the web margin. It contains the entire lymphatic supply and one blood capillary plexus. A second similar set of blood vessels, the plantar group, underlies the lymphatics. From this illustration it is possible to get a rough idea of lymph capillary and blood capillary areas in a given block of tissue. When the lymphatic meshwork was traced, cut out and weighed, a figure, 0.6399 gram, was obtained. A cut-out of the dorsal capillary plexus weighed 0.3133 gram. This figure must be multiplied by 2 in order to account for the plantar capillaries, which gives 0.6266 gram. There is a striking similarity of area. In another region, where there was no suggestion of lymphatic rupture, but where distention certainly existed, the lymphatic figure, for a given area, was 0.1507 gram and the corresponding blood vessel figure 0.0880 gram. These findings assume that the lymphatics are cylindrical, which is probably true in the injected condition. In the normal web they are certainly flattened ovals which tend on filling to become cylindrical. The lymphatic capillaries are astonishingly distensible. In preparations such as those shown in figure 1, one may increase the injecting pressure and observe lymphatics with an initial diameter of 70 μ become a third wider, but this distention need not be attended by leakage of particles. Under normal circumstances the endothelial coat of these vessels is extremely thin. When stretched it must reach an extreme degree of tenuity, but stretching does not produce definite openings unless very excessive. In a former paper (1931) as an explanation of the ease with which lymphatics are entered, the authors remarked:

> We are inclined to believe that motion or massage, any movement of the tissue in which they exist, causes the lymphatics to lose their anatomical integrity of wall and to become continuous with the tissue spaces. Lymphatic capillaries possess an extreme tenuity of wall and if attached on the outside to surrounding structures might readily be pulled open on motion of the part and yet in entire quiescence exist as closed tubes. In order to explain the ready entrance of particles, of solutions containing protein, and so forth, into the lymphatics, we have thus postulated a state of affairs for lymphatics in general not different from that actually described by MacCallum (1903) for the diaphragmatic lymphatics.

This conception of the formation of actual stomata is out of accord with our observations on the web of the frog, and with many experiments on the subcutaneous lymphatics in the foot of the dog. Material entering lymphatics, except in the diaphragm, apparently invariably passes through a membrane and cannot take advantage of openings into the tissue spaces, since such openings do not exist. If one has injected lymphatics under observation and gradually increases the injecting pressure, the vessels increase slowly in diameter and finally rupture. There is no escape through a system of preformed clefts in the wall or at many points between cells. Material leaves the lymphatics in spurts which appear at random in the web. A point at present entirely inexplicable to us is the fact that graphite particles, placed outside lymphatics, begin to enter them at once if the part is massaged or moved, or if there is a heavy drift of fluid into the lymphatics, as occurs in the early stages of inflammation swelling. Yet if graphite in an artificial lymph is injected into lymphatics, the particles are not forced through the wall when the pressure is raised. They get outside when a definite rupture occurs, the accident having exactly the same appearance as a capillary hemorrhage.

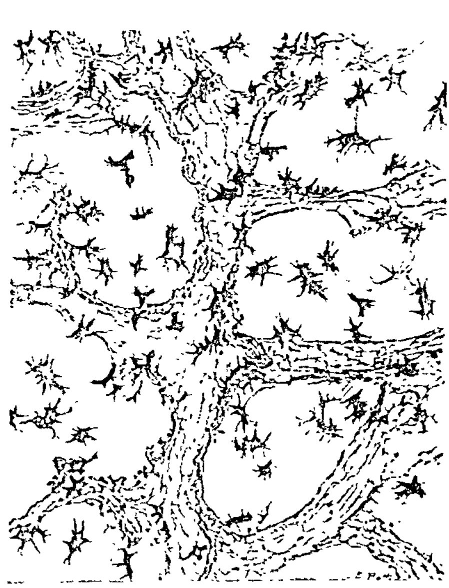

Fig. 4. Silver nitrate preparation of the web lymphatics. $\times 43\frac{1}{3}$.

Figures 4 and 5 show something of the delicacy of the lymph capillaries in the web. There is no suggestion of structure beyond the ill-defined living membrane, nothing capable of causing spontaneous contraction or relaxation. In many experiments we have watched the lymphatics during the entire day, making repeated photographs of the same area, and have traced the lymphatic outlines from these photographs. Over long periods of time the diameter of individual vessels occasionally changed slightly, but these changes were so slow as to be inconsistent with a spontaneous process of contraction. Mechanical stimulation, faradization, and heavy intravenous injections of adrenin, were all ineffectual in causing change in diameter. That the adrenin left the blood capillaries and reached the walls of the lymphatics was shown by contraction of melanophores lying directly over the latter vessels—melanophores in the position of the two seen in figure 5.

Our entire failure to obtain evidence of contractile powers on the part of the lymphatic endothelium contrasts with certain of the experiences

summarized by Carleton and Florey (1927) in a paper which combines their direct physiological and histological observations upon the lacteals of a variety of animals. They remark,

One species—the squirrel—has a very scanty supply of smooth muscle. It is only in vessels above 300μ that any can be detected. In the smaller lacteals of the cat also no smooth muscle can be found. These vessels have been seen to contract when stimulated by a strong faradic current or mechanically. It must be confessed that neither of these stimuli are physiological, and that the vessels without muscle nuclei do not contract to such drugs as adrenalin or pituitrin. Nevertheless, it would appear that one is dealing with vessels which are capable of contraction,

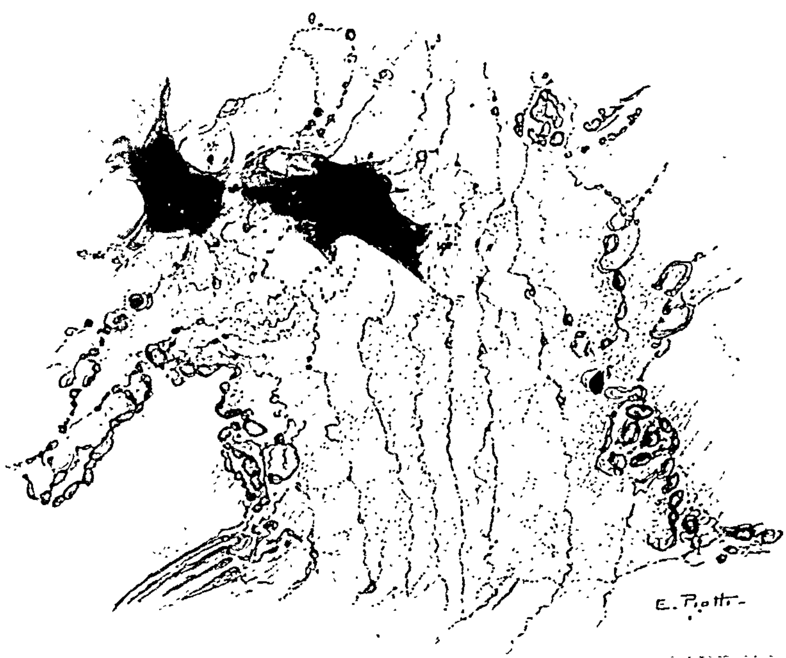

Fig. 5. Silver nitrate preparation of part of a web lymphatic. × 600

though possessed only of an endothelial wall. There can be no question of the presence of such cells as the Rouget type to account for contraction. Smooth muscle, after the most careful search, has not been found.

Lieben's observations (1910) on contraction of lacteals in the mouse and rat apparently are confined to vessels containing smooth muscle. Tarchanoff (1874) reported that lymphatics in the frog's web underwent the same sort of narrowing when stimulated electrically as did the blood capillaries. This narrowing Tarchanoff described as due to the swelling of cells at different points in the wall, and in addition to this there was a general reduction in lumen which he was unable to explain. His observations are the only ones describing spontaneous contraction of lymphatics other than those in the mesentery. Again, as in the case of Carleton and Florey, strong elec-

trical stimulation was required. We have found faradization just as inefficacious as other forms of stimulation, and have tested it in preparations which would certainly have displayed change in size were it possible to bring this about. It is our conviction that under normal circumstances the finest lymphatics, those possessing no lining save endothelium, change size passively in response to changes occurring in the surrounding tissues. It is the usual idea that edema of a part must cause compression of lymphatics. This is not the case. It would be so in the web of the frog if during edema formation the part was inclosed in a rigid plaster matrix. Then increase in intercellular fluid would close off yielding structures such as the lymphatics. But if the part swells freely the lymphatics are opened. They are simply endothelial-lined clefts between the cells of the part dilated with the oversupply of fluid in the part.

SUMMARY

1. New methods are described for vital injection of the lymph capillaries in the web of the frog.
2. The surface areas of lymph and blood capillaries are found to be much the same.
3. No evidence could be obtained indicating spontaneous contractile power in the lymph capillary endothelium.
4. The lymph capillaries are capable of showing changes in calibre, but in normal life these are brought about passively.
5. There is no evidence of normal clefts or stomata in the lymph capillary walls.

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PHYSIOLOGY OF THE CORPUS LUTEUM

VIII. Interrelationship of Oestrin and the Corpus Luteum as Determined by their Effects in the Adult Rabbit

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We have previously described[1] the production of active corpus luteum extracts which, when injected into adult castrated rabbits, cause the production of progestational proliferation of the uterus, and when injected into mated animals castrated eighteen hours after mating, will maintain pregnancy to term. The active substance responsible for this we have named progestin, since that name seems to best describe its physiological properties. We have also shown that this substance has practically no effect on the immature rabbit's uterus, unless the uterus has first been brought into a state of maturity by the preliminary injection of small amounts of oestrin.[2] These observations have been confirmed by Clauberg (1930 a, b, c), Fels (1931), Hisaw and Leonard (1930), Robson and Illingworth (1931), and Fremery, Luchs and Tausk (1931).

It has also been shown by Hisaw (1929) that for corpus luteum extracts to relax effectively the guinea pig's symphysis the pigs must be either in natural or artificially produced heat. The substance responsible for this relaxation has been named relaxin and has been shown to be different chemically from progestin in that it is ether insoluble. Hisaw and Leonard (1930) have also made the observation that progestin has no effect on rabbits which have been castrated for some time, and furthermore, that doses of oestrin sufficient to keep the animal in heat will inhibit the effect

[1] Previous papers of this series are listed in the bibliography as follows: I, Corner, 1928; II, Corner and W. M. Allen, 1929; III, Allen, W. M. and Corner, 1929; IV, Goldstein and Tatelbaum, 1929; V, W. M. Allen, 1930a; VI, W. M. Allen, 1930b; VII, Allen, W. M. and Corner, 1930.

[2] Throughout this paper the use of the word oestrin will be used when speaking in general of the oestrogenic hormone. This general term seems to best describe this hormone and should be more widely used. Menformon (Laqueur, Dingemanse and Kober, 1930), Theelin (Thayer, Veler and Doisy, 1930) and Progynon (Butenandt, 1929) are terms used by these investigators to designate the crystalline oestrogenic hormone which each has independently isolated. Other proprietary names for the same hormone in less purified state are, Amniotin and Estrogen.

of progestin. These seem to have been isolated observations and do not contain definite information regarding quantitative relationships between oestrin and progestin. On the other hand, Courrier (1928, 1930) has shown that in the normal, pregnant rabbit large doses of oestrin will prevent the development of proliferation and the normal growth of the embryos if given during the first five to six days after mating. If the injections are made from the sixth to eleventh day, proliferation is not affected but the embryos are lost.

Others have shown also that oestrin injections during pregnancy will cause abortion or resorption of embryos. (Rat (Smith, 1926); guinea pig (Kelly, 1931); mouse (Parkes and Bellerby, 1926).)

All of these observations seem to indicate that oestrin when given in large amounts may have a detrimental effect on the continuance of normal pregnancy and suggest that this effect may be due to interference with the activity of the corpus luteum. It seems necessary, therefore, to investigate the interrelationship of oestrin with progestin, since if oestrin inhibits the action of progestin it makes invalid any tests for the presence of the latter in oestrin-containing extracts such as human placenta or pregnant urine. It is quite essential from both the physiological and the economic standpoints to find out whether there are sources for progestin other than the corpus luteum.

In the experiments to be described subsequently the unit of oestrin[3] used for all extracts, with the exception of the Estrogen given to X-337 and X-350, was the minimum amount necessary to bring approximately 50 per cent of 20 castrated rats into full heat as determined by vaginal cornification, following three injections spaced 12 hours apart. With the exception of rabbit X-350 all rabbits in the first group were injected with the same extract of oestrin; thus making the difference between the two groups significant. The unit used in the standardization of the Estrogen was the minimum amount necessary to bring 3 of 4 castrated rats into full heat when given 3 injections spaced 12 hours apart. The unit of progestin has been previously defined (Allen, 1930a). It is the minimum amount which produces a +++ or ++++ proliferation in the adult female rabbit castrated the day following mating, and injected for 5 days, the first injection being made on the day of castration and autopsy being performed on the 6th day after mating and one day after the last injection.

The first part of the investigation was carried out to ascertain whether oestrin will inhibit the action of progestin-containing extracts as determined by microscopical study of the endometrium. In table 1 are recorded the results found from the injection of oestrin into normal rabbits (X-350, X-375, X-376) during the first five or six days of pregnancy and those

[3] The Estrogen and the Theelin were furnished by Parke, Davis & Co. The Amniotin was supplied by Dr. J. F. Anderson of Squibb & Co.

obtained by injection of oestrin plus 3 rabbit units of progestin over a period of five days into rabbits, castrated 18 hours after mating (X-362, X-373, X-382). In all cases autopsy was performed the day after the last injection was made.

It is seen immediately that a dose of oestrin (675 r.u.) when combined with three times the minimal dose of progestin prevents the development of proliferation but that the same dose of oestrin does not inhibit completely the occurrence of proliferation in the normal, mated rabbit. It should be stated that three times the minimal dose of progestin necessary to produce a +++ proliferation will always produce complete proliferation in the adult recently castrated rabbit. This dose of oestrin, even though

TABLE 1

Effect of oestrin in early pregnancy compared with the effect of oestrin plus progestin in castrates

RABBIT NUMBER	TYPE AND AMOUNT OF EXTRACT USED	DURATION OF TREATMENT	RESULTANT PROLIFERATION	EMBRYOS
X-350	400 r.u. Estrogen	2–6 da. p.c.	++++**	2 abnormal
X-375	675 r.u. oestrin† from preg. urine	1–5 da. p.c.	+++	None
X-376	675 r.u. oestrin from preg. urine	1–5 da. p.c.	+++	None
X-362	675 r.u. oestrin.* 3 rabbit units progestin	1–5 da. p.c.	None (congestion)	None
X-373	Same as X-362	Same as X-362	None (much congestion)	None
X-382	Same as X-362	Same as X-362	None (much congestion)	None

* Castrated 14–18 hours after mating.

† Made in this laboratory by extracting the urine of pregnant women with benzene.

** For illustrations showing the standard degrees of proliferation as indicated by these signs, see W. M. Allen, This Journal 1930a.

p.c.—post coitus.

r.u.—rat units.

not sufficient to prevent the natural occurrence of proliferation does affect the embryos, for in two cases none were found and in the third case where 400 r.u. were given but two abnormal ones were found. This group of experiments indicates that oestrin will definitely prevent the action of potent corpus luteum extracts which contain progestin, and that the dose necessary to inhibit three rabbit units of progestin is not enough to inhibit completely the activity of the progestin produced by the rabbit's own corpora lutea. The loss of embryos in those receiving both progestin and oestrin cannot be logically explained either by the lack of proliferation or the surgical trauma due to the öophorectomy, since in the routine testing

of corpus luteum extracts (Corner and Allen, 1929; Allen, 1930a), where ordinary care is taken during the operation, normal embryos are obtained if the extract is active, and degenerated ones if the extract is inactive. It is even more difficult to explain their loss in those treated only with oestrin, for in these cases there was no operation and proliferation was present at autopsy.

The results obtained from this group agree essentially with the finding of Tausk, de Fremery and Luchs (1931) that 980 mouse units of Menformon inhibited completely one rabbit unit of progestin.[4] Actual comparisons cannot be made, however, because their rabbit unit is based on standardizations using immature rabbits previously treated with Menformon as devised by Clauberg (1930c), and because the Menformon was standardized as mouse units rather than rat units. The observations, regardless of unimportant differences in the method of standardization, indicate that progestin can be inhibited by extracts containing oestrin. They also state that they have been unable with the doses given to inhibit the action of the corpus luteum in the normal pseudopregnant animal.

Summary. The ability of 3 rabbit units of progestin to produce progestational proliferation in the castrated rabbit is completely inhibited by the simultaneous injection of 675 r.u. of oestrin. The same dose of oestrin given to a normal rabbit during the first five days after mating is insufficient to prevent the proliferation produced by the rabbit's own corpora lutea.

The next step was to determine whether the proliferation produced in the normal rabbit by action of her own corpora lutea could be overcome by larger doses of oestrin than those given to rabbits X-350, X-375, X-376. It seemed quite probable that such would be possible and while this work was being done Courrier (1930) published observations showing that doses of 500–700 r.u. given during the first five days after mating would prevent the development of proliferation. Previously (1928) he had shown that doses of 230 r.u. given from the fifth to eleventh day had no effect on the proliferation but did cause the disappearance of the embryos.

Preliminary observations showed that 750 r.u. of oestrin given during the first five days after mating prevented the development of proliferation, but injection during the next five days did not destroy the proliferation which was already present at the time when injections were started. These

[4] These findings are in agreement with the recent work of Leonard, Hisaw and Fevold (This Journal, 1932, c, 111). Their paper appeared after the author's manuscript was sent to the publisher so that it could not be discussed in the body of the paper. They found that 25 r. u. of Theelin inhibited completely 1 rabbit unit of progestin but that 2, $3\frac{1}{3}$, $5\frac{1}{3}$ rabbit units respectively were not completely inhibited by this amount of Theelin. This work together with that of the author confirms and extends that of Tausk, de Fremery and Luchs (1931).

experiments directly confirmed those of Courrier and immediately suggested the further investigation of the subject to ascertain what was actually happening in both uterus and ovaries, especially with reference to the corpora lutea in those injected during the first five days and to the embryos in those injected during the next five days.

In this group of experiments (table 2) animals were injected with from 80 to 1000 rat units of oestrin. In the first five (X-337, X-350. X-375, X-376, X-619) injections were made during the first five days after mating (except X-350 which was injected from the second to sixth days inclusive)

TABLE 2

Effect of oestrin in the first few days of pregnancy

RABBIT NUMBER	PERIOD OF INJECTIONS	TOTAL DOSAGE OESTRIN GIVEN	ULTIMATE DISPOSAL	PROLIFERATION	REMARKS
X-337	1–5 da. p.c.	80 r.u. Estrogen	Autopsy 6 da. p.c.	+++	
X-350	2–6 da. p.c.	400 r.u. Estrogen	Autopsy 7 da. p.c.	++++	2 abnormal embryos
X-619	1–5 da. p.c.	500 r.u. Theelin	Biopsy 6 da. p.c.	+++	
			Autopsy 10 da. p.c.	+++	1 large implantation
X-375	1–5 da. p.c.	675 r.u. oestrin*	Autopsy 6 da. p.c.	+++	Congestion
X-376	1–5 da. p.c.	675 r.u. oestrin*	Autopsy 6 da. p.c.	+++	Congestion
X-604	2–5 da. p.c.	700 r.u. Amniotin	Autopsy 6 da. p.c.	+	Congestion
X-398	1–5 da. p.c.	750 r.u. Theelin	Biopsy 6 da. p.c.	None	Congestion
			Autopsy 13 da. p.c.	None	No necrosis
X-606	1–5 da. p.c.	1,000 r.u. Amniotin	Autopsy 6 da. p.c.	None	Marked congestion No necrosis
X-614	1–5 da. p.c.	1,000 r.u. Theelin	Biopsy 6 da. p.c.	None	Marked congestion
			Autopsy 10 da. p.c.	None	No necrosis

* Made from the urine of pregnant women.

and autopsy or biopsy was performed the day following the last injection. In each of these five cases normal proliferation and corpora lutea of normal appearance were present. No embryos at all were found in X-337, X-375, and X-376 and in X-350 two abnormal ones (3 times normal size with abnormal-looking embryonic areas) were found. In X-619 at autopsy on the 10th day there was one large (1.5 cm.) implantation site which upon section was found to have a normal placenta but an embryo with large blastocystic cavity. Injections were made in the remaining animals of this group during the same period of time but larger doses were given (700–1000 r.u.). Animals X-604 and X-606 were autopsied on the sixth day

and no proliferation was found, embryos were absent and normal corpora lutea were present in the ovary. Since in these two animals normal proliferation was absent even though the corpora lutea appeared normal both grossly and microscopically, the next two animals (X-614, X-398) were submitted to laparotomy on the sixth day and one ovary and a biopsy of the uterus removed. Autopsy on these was then delayed until the tenth day in order to ascertain whether the normal appearing corpora lutea present in the remaining ovary might not cause the production of proliferation from the 6th to the 10th day after the injections of oestrin were discontinued. Such was not the case, for at autopsy the uteri of both showed no proliferation even though the remaining ovary in each contained numerous corpora lutea which appeared normal both in gross and on microscopic study. Furthermore, there was no tissue-necrosis, thus differentiating them from those injected from the 5th to 9th days as will be described subsequently.

The uteri of all animals in this group in which the dose was large enough to prevent the development of proliferation showed marked congestion and were of a dark purple color, when observed in the fresh state. With the larger doses of oestrin the congestion was so extreme that the uterus was almost as blue as venous blood. The congestion was limited to the uterus and to a lesser extent the vulva. The ovarian and tubal vessels were not engorged, and the distinction between uterus and cervix was well demarcated, the cervix being of normal, pink color. Microscopically, there was extreme dilatation of the endometrial vessels, especially those immediately below the epithelium. In no case was there any tissue-necrosis, blood in the lumen or definite evidence of true extravasation. These findings are in contrast to those of Clauberg (1930c) who found extravasation in immature rabbits treated with menformon (350 mouse units in 20 days), but the experiments are not directly comparable.

Summary. In this group, therefore, oestrin when given in large enough doses (1000 r.u.) during the first five or six days after mating, prevented the development of proliferation and caused the disappearance of the embryos but did not prevent the development of normal-looking corpora lutea. Furthermore, even though the corpora persisted in the normal state from the fifth to tenth day, no proliferation was produced after its development had been prevented by the injection of oestrin.

The next group of animals (table 3) was injected with doses of oestrin comparable to those given in X-398, X-604, X-614 of the preceding group, with the difference that they were injected from the fifth to the ninth day after mating and were autopsied on the tenth day. In fact, these animals were injected in paired experiments, i.e., X-398 and X-600 were injected from the same lot of Theelin and at the same time; X-606 and X-610 from the same lot of Amniotin, etc. They were conducted in this manner to

eliminate as far as possible indeterminable variations and to make the results obtained in those injected during the first five days all the more comparable with the results from those injected during the second five days.

In X-600, which received 750 r.u. of Theelin, there were 3 uterine enlargements, one of which upon serial section showed typical development of the two endometrial folds adjacent to the mesometrium, with characteristic changes in the superficial epithelium and perivascular regions. (See the plates of Duval (1892) for normals.) Embryonic structures were absent and there was extreme vacuolar degeneration and nuclear shrivelling in the perivascular regions, but no disintegration of the endometrium. Between the implantation sites the proliferation appeared to be identical with that of a 13-day pregnancy or pseudopregnancy. In X-610 and X-616, which received 1000 r.u. each of Amniotin and Theelin respectively, there were numerous abnormally small implantation sites. Upon incising the uterus of each between the enlargements a thin, grayish-brown fluid exuded. Histological study of these specimens revealed a very striking picture. Extreme tissue-necrosis with actual sloughing of the endometrium was present in many places, and in the region of the implantation sites such extensive congestion and degeneration were present that the placental structures were hardly recognizable (fig. 2). Between the enlargements, where the degeneration was not too extensive, normal proliferation was still present in some places. The endometrial vessels were distended with blood, and in many there were definite, laminated thrombi (fig. 3). Despite these thrombi, there were very few regions in which extravasation of blood was found. In these latter two animals remnants of the embryos

TABLE 3

Effect of oestrin during the first five days of pregnancy compared with the effect of similar doses in the second five days

RABBIT NUMBER	PERIOD OF INJECTIONS	TOTAL DOSAGE OESTRIN GIVEN	ULTIMATE DISPOSAL	PROLIFERATION	REMARKS
X-398	1–5 da. p.c.	750 r.u. Theelin	Biopsy 6 da. p.c.	None	Cong. no necrosis
			Autopsy 13 da. p.c.	None	No necrosis
X-600	5–9 da. p.c.	750 r.u. Theelin	Autopsy 10 da. p.c.	+++	Dead embryos Path. placentae
X-606	1–5 da. p.c.	1,000 r.u. Amniotin	Autopsy 6 da. p.c.	None	Cong. no necrosis
X-610	5–9 da. p.c.	1,000 r.u. Amniotin	Autopsy 10 da. p.c.	Some	Much necrosis, path. placentae, dead embryos
X-614	1–5 da. p.c.	1,000 r.u. Theelin	Biopsy 6 da. p.c.	None	Cong. no necrosis
			Autopsy 10 da. p.c.	None	No necrosis
X-616	5–9 da. p.c.	1,000 r.u.	Autopsy 10 da. p.c.	Some	Much necrosis

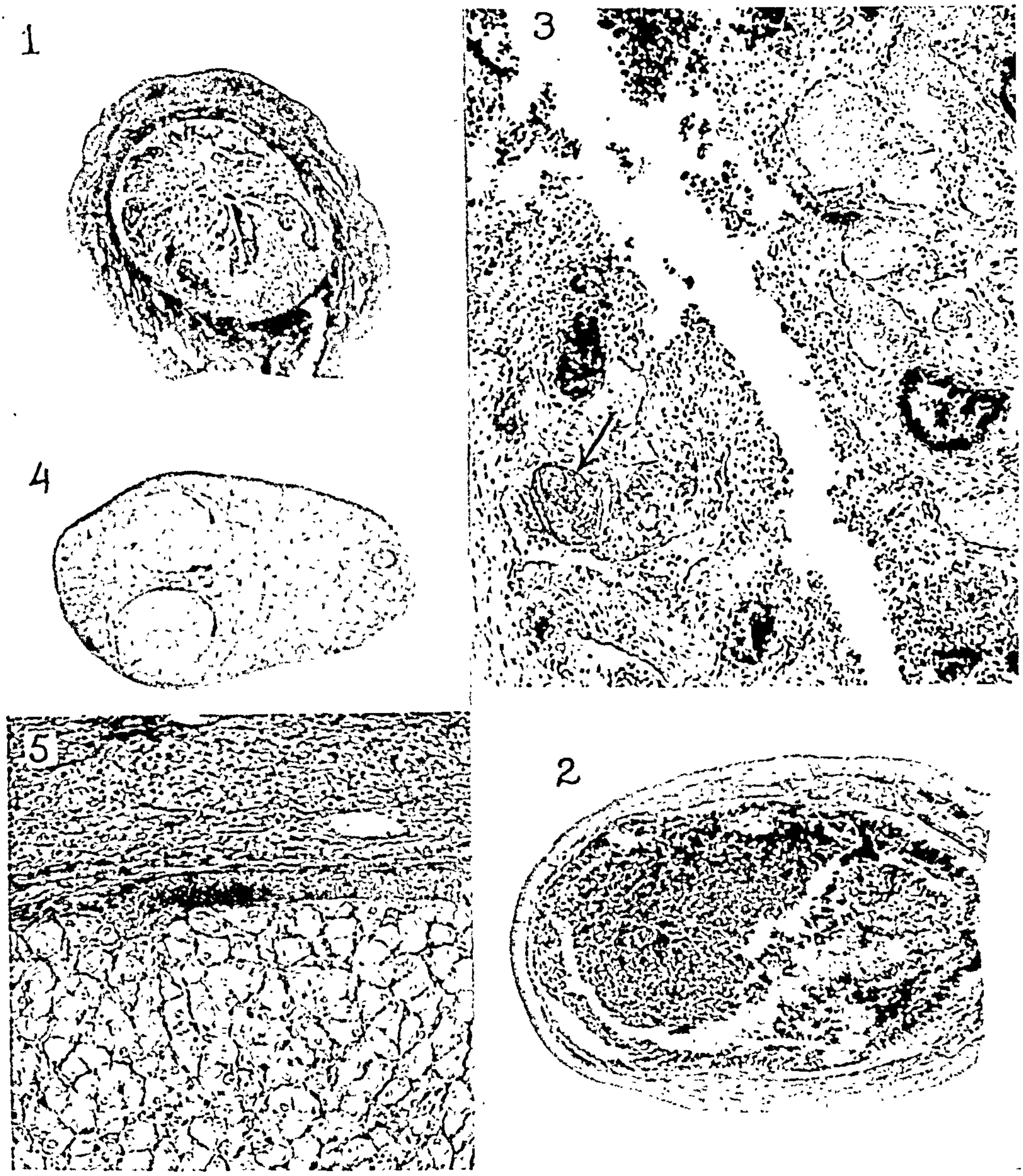

Fig. 1. Photograph of a section taken between placental sites of rabbit X-616. Note the degeneration of the glands and the debris in the lumen. × 5.5.

Fig. 2. Photograph of a section through a placental site of rabbit X-616. The placental site is completely degenerated, the entire endometrium is necrotic and the lumen is filled with debris. The muscular layers are not involved. × 5.5.

Fig. 3. Higher magnification of the same section as that shown in figure 1. Note the cellular debris, engorged venous sinuses, and the ante-mortem thrombus at *arrow*. The diagonal white space through the section is not an artifact but the crypt of a gland, the epithelium of which has completely sloughed off. × 100.

Fig. 4. Photograph of a section of ovary from rabbit X-616 showing corpora lutea of normal size. × 5.5.

Fig. 5. Higher magnification of section shown in figure 4. With ordinary hematoxylin and eosin stains the cellular elements appear to be normal.

The rabbit from which all of these photomicrographs were made was injected with a total of 1000 rat units of Amniotin from the fifth to ninth days after mating. Autopsy was performed on the tenth day, the day after the last injection. All injections were subcutaneous.

were still identifiable. The ovaries obtained from these animals all contained numerous corpora lutea which appeared normal both grossly and microscopically (figs. 4, 5).

Summary. In this group the injection of 1000 r.u. of oestrin from the 5th to 9th days after mating produced extensive degeneration of the endometrium and death of the embryos, but caused no cytological changes in the corpora lutea.

From a comparison of X-606 and X-614, injected with 1000 r.u. during the first five days, and X-610 and X-616, injected with 1000 r.u. during the second five days, it is seen that 200 r.u. per day in the former caused extreme congestion and prevented the development of proliferation but produced no visible degenerative changes whereas the same daily dose in the latter caused extensive endometrial necrosis. The first gross difference between the uteri of these two groups which comes to mind is that in the former, injections were begun before proliferation had appeared, whereas in the latter, proliferation (normally well developed at the fifth day) was already present when injections were started. It seemed worthwhile therefore to inject animals with 200 r.u. per day for the first three days with biopsy or autopsy on the sixth day to see how long it is necessary to give oestrin to prevent development of proliferation. Consequently, X-611 and X-615 (table 4) were injected with 200 r.u. daily of Amniotin and of Theelin respectively, for the first three days following mating. X-611 was autopsied on the 6th day. The uterus was found to be normally proliferated and the ovaries to contain normal corpora lutea. No embryos were found in the uterus (where they should be on the 6th day) but from one tube several small, undeveloped embryos were obtained. X-615 was

TABLE 4

Effect of oestrin at other times during pregnancy

RABBIT NUMBER	PERIOD OF INJECTIONS	TOTAL DOSAGE OESTRIN GIVEN	ULTIMATE DISPOSAL	PROLIFERATION	REMARKS
X-611	1–3 da. p.c.	600 r.u. Amniotin	Autopsy 6 da. p.c.	++++	Dead blastocysts in one tube
X-615	1–3 da. p.c.	600 r.u. Theelin	Biopsy 6 da. p.c.	++	
			Autopsy 10 da. p.c.	++	No embryos
X-625	4–5 da. p.c.	400 r.u. Theelin	Biopsy 6 da. p.c.	+++	
			Autopsy 13 da. p.c.	+++	2 embryos
X-626	4–5 da. p.c.	400 r.u. Amniotin	Biopsy 6 da. p.c.	++++	No embryos, slight degeneration
			Autopsy 13 da. p.c.	+++	
X-605	22–26 da. p.c.	750 r.u. Amniotin	Autopsy 27 da. p.c.	All fetuses dead	

submitted to laparotomy on the 6th day and one ovary and a segment of the corresponding cornu were excised, care being taken not to disturb the other ovary or cornu. At autopsy on the 10th day no implantation sites were present in either cornu. Both the ovary removed on the 6th day and the one obtained at autopsy on the 10th day contained normal corpora lutea. The uterus likewise showed slightly subnormal proliferation at both times.

From these and preceding experiments it is seen that 200 r.u. per day for the first three days after mating does not prevent proliferation, whereas 200 r.u. per day for the first five days does prevent proliferation. The next two animals (X-625, X-626) (table 4) were injected with 200 r.u. daily of Theelin and Amniotin respectively on the 4th and 5th days after mating. They were both submitted to laparotomy on the 6th day and a segment of uterus was excised. Normal appearing corpora were observed in both ovaries. The endometrium of one (X-626) showed slight degeneration with sloughing of the epithelium but without hemorrhage. At autopsy on the 13th day the uterus of one (X-625) contained 2 apparently normal implantation sites. In this one normal proliferation was present and the corpora lutea were normal.

Summary. Injection of 200 r.u. daily of oestrin for the first three days after mating does not prevent the development of proliferation but does cause disappearance of the embryos. Injection of 200 r.u. daily on the 4th and 5th days after mating produces slight degenerative changes in the proliferated endometrium.

An incidental experiment was the injection of 750 r.u. over a period of 5 days of Amniotin to one rabbit (X-605) beginning at about the 20–22 day of pregnancy. Autopsy the day after the last injection showed that all fetuses were dead and that death was due to separation of the placentae. Microscopical study of the placentae revealed extensive infarct formation and necrosis.

Discussion. The fact that only moderate doses of oestrin (675 r.u.) completely inhibit the ability of progestin to produce proliferation is of considerable importance in that it makes the assay of extracts for progestin invalid if they contain similar amounts of oestrin (table 1). For example, if, in the assay of a liter of urine for progestin, as much as three rabbit units of progestin were present, its presence could not be detected without first removing the oestrin, because this amount of urine might be expected to contain 1000 r.u., enough to inhibit the progestin. For this reason observations that the placenta contains no progestin are not proof necessarily that there is none there. In fact, in our original publication giving a method of preparation of an active substance from the corpus luteum, (Corner, G. W. and W. M. Allen, 1929) we stated that we had been unable to produce active extracts from the placenta but felt that possibly there

might be an inhibitory action of the oestrin present on the progestational substance (progestin). Fellner (1913) indicates that he was able to produce proliferation in some of his immature rabbits with placental extracts. However, the proliferation might have been due to the rabbits' own ovaries since the method of preparing the extract (warm saline) given to the animal from which the illustration was made does not preclude the possibility of the extract containing so-called "anterior lobe" hormones. The converse of the effect of oestrin on the assay of progestin, i.e., the effect of progestin on the assay of oestrin, has been worked out by Angelvitz and Sterling (1930). They found that the injection of 1 rabbit unit of progestin had no appreciable effect on the standardization of oestrin-containing compounds.

Little significance probably can be attached to the observation that it takes more oestrin to inhibit the development of proliferation by the rabbit's own corpus luteum than it does to inhibit that produced by an extract containing progestin, since it seems quite likely that progestin produced by the rabbit's own corpora may be more readily available to the uterus than progestin injected subcutaneously in an oily medium.

With regard to the effect of oestrin on the corpora lutea, it need only be said that even with doses sufficient to prevent completely the development of proliferation, the corpora lutea grew to normal size and by ordinary hematoxylin and eosin stains appeared to be normal microscopically. These observations are in accord with those of Kelly (1931), who found no microscopical abnormalities in the ovaries of pregnant guinea pigs injected with as much as 600 r.u. of oestrin even though this dose caused placental degeneration and death of the fetuses. Courrier (1930) using rabbits also found no changes in the corpora with doses of 500–700 r.u. However, the fact that they appear normal does not necessarily mean that they are functional. Deanesly (1931) has shown that injections into pseudo pregnant mice of 3–10 mouse units of oestrin over a period of 36 hours during the first half of pseudo pregnancy causes variable changes in the lipoidal deposits stainable with osmic acid.

It seems quite likely that the doses given are far in excess of those found in the normal rabbit at any time. The fact that proliferation can be prevented and also that actual endometrial necrosis and placental degeneration can be produced by oestrin without producing any demonstrable change in the corpus luteum indicates that these changes have no counterpart in the normal cycle, for, in the normal animal, when proliferation disappears at the end of pseudopregnancy it is accompanied by cytological signs of regression such as vacuolar changes in the cytoplasm of the corpora lutea. The significance of the endometrial necrosis produced in those (X-610; X-616) injected from the 5th to 9th days is dubious. Bleeding has been produced in the castrated dog during injections of oestrin (Kunde

et al., 1930; Meyer and Saiki, 1931) and menstruation-like bleeding occurs in the castrated monkey following the cessation of oestrin injections, but in these two animals similar phenomena are observed at definite times during the normal cycle. In the rabbit,[5] however, no such phenomena are observed at any time during the cycle. The observations therefore cannot be interpreted as representing any phase of the normal cycle. It is of interest that a dose of 200 r.u. per day for 5 days causes necrosis of endometrium when given from the 5th to 10th days, whereas, when given during the first 5 days it seems to have no such effect, the result during that time being congestion and prevention of proliferation. Why should the same dose cause such different responses at such closely related times of the cycle? It would seem that to produce this necrosis the uterus must be proliferated, and therefore that progestin may have some rôle. If it be due to toxicity then Theelin in large enough doses is a toxic drug with its toxicity probably localized chiefly on the uterus. This result can not reasonably be assigned to impurities since Theelin is a crystalline chemical compound and 1000 r.u. weigh only about 0.3 mgm. (3000 r.u. per milligram, Thayer, Veler and Doisy, 1930).

The mechanism by which the oestrin causes the death of the embryos in these experiments is obvious in all those cases where the doses were sufficient to prevent the development of proliferation or to cause pathological changes in the placentae. In these cases their death may reasonably be attributed to faulty nutrition. However, the absence of embryos in those animals in which the dose of oestrin given caused neither the absence of proliferation or endometrial necrosis is not so readily explained. It is more than likely that failure of implantation may be obtained with much smaller doses of oestrin than those given but this phase of the problem has not been studied as yet in the rabbit. Kelly (1931) has shown that oestrin (75–150 r.u.) given to the guinea pig in early pregnancy (12–17 days) regularly caused abortion and that doses of as little as 10 r.u. given during first 2 days after mating caused failure of conception. The prevention of conception is not likely to be due to any failure of the corpus luteum since in the rabbit as above mentioned proliferation is normally present and yet the embryos are lost.

The important observations of Reynolds (1931a, 1931b), however, give a possible explanation. He has found that normally at oestrus the uterus exhibits marked rhythmical contractions and that oestrin injections of as little as 2 r.u. per kilogram of body-weight cause increased motility of the uterus when given to a recently castrated rabbit. (The observations were made by means of intravitam balloon studies on uterine fistulae.) Even moderate doses, therefore, may very likely cause sufficient motility to

[5] For a detailed study of reproduction in the rabbit see the monograph by Hammond and Marshall (1925).

prevent the normal passage of the ova through the tubes or to get them into the uterus too soon (before proliferation has developed). However, in the pseudopregnant rabbit the uterus begins to lose its reactivity to oestrin quite early and during the period from the third or fourth to fourteenth days it is non-reactive to as much as 1090 r.u. of Theelin per kilogram of body weight. The ready ease with which pregnancy can be terminated in the first few hours or days perhaps can be explained on increased or abnormal motility of the uterus. Abnormal motility, however, probably cannot be used as an explanation later in pregnancy, but at this time such an explanation is unnecessary because with big enough doses recognizable, cytological changes of a degenerative type are evident in the uterus.

CONCLUSIONS

1. Active progestin-containing extracts lose their ability to produce progestational proliferation in the castrated rabbit's uterus when they are given simultaneously with sufficient amounts of oestrin; 675 r.u. of oestrin inhibit completely 3 rabbit units of progestin.
2. Large doses of oestrin (1000 r.u.) given during the first 5 days after mating prevent the development of proliferation but do not prevent the development of normal appearing corpora lutea.
3. Large doses of oestrin (1000 r.u.) given during the second five days after mating cause extensive endometrial degeneration with sloughing of the endometrium, but produce no demonstrable changes in the corpora lutea. The placentae start to form but are very abnormal.

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THE PHYSIOLOGY OF DRAINAGE OF NASAL MUCUS

III. Experimental Work on the Accessory Sinuses

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A description of the mechanics of drainage of the frontal sinus of a dog is presented in this paper. It is the third of a series on the physiology of the nose and sinuses. A general description as to this method of drainage was given in a previous contribution, but the details had not been worked out at that time. The results cannot be directly applied with certainty to the sinuses of man, but it is highly probable that the principles of drainage are very similar.

Very few studies of this nature seem to have been made. Lucas (1931) made some observations on the maxillary sinuses of the monkey (Macacus rhesus). He reported the presence of spiral drainage similar to that found in sinuses of the dogs used in the present study. Yates (1924) made observations on the effects of different types of secretion on ciliary activity.

Twenty-seven dogs were used in my experiments. The sinuses of most of the animals were opened for inspection six or eight times. Not all the tests were made in both sinuses at the time of every operation on each dog. That would have required several hours of anesthesia each time. Instead, a few data at a time were obtained from each dog, as convenient.

Description of sinus. The frontal sinuses of different dogs vary greatly in size and shape. In general the volume is 5 to 7 cc. A sinus is roughly oval in shape, with the ostium at the anterior end (fig. 1). Each sinus is separated from its fellow of the opposite side by a septum which makes the mesial wall of both straight for a distance. Usually there is a more or less prominent, approximately horizontal ridge in about the same plane as the plane of the frontal bone that divides the sinus into a more superficial, larger chamber, and a smaller, deeper chamber. The latter may be very deep and somewhat funnel-shaped. The ostium lies rather high in the more superficial chamber. In the tests, the time required to move material out of the sinus, up the steep slope from the bottom of the deep chamber to the ostium was measured (fig. 1). There are often several recesses in the posterior part of the superficial chamber that are formed by incomplete partitions. The roof is rather flat. Anteriorly,

there is a peculiar structure shaped like a leaf of a water-lily, that lies *mesial to the ostium. It seems to originate* in the ostium. It is extremely thin and is covered with epithelium on both sides. It usually lies flat against the wall, as though molded there. A very narrow edge is often turned up at right angles at the margin. An ethmoid sinus often protrudes into the anterior part of the frontal sinus, like a bulb.

TECHNIC. Ether was administered by tracheal tube. The standard operation which was used was simple. The frontal bone was laid bare through an incision in the median line, and the roofs of both sinuses were removed more or less completely by means of chisel and rongeur.

The lines of drainage were studied by watching the movements of drops of India ink placed here and there on the interior surfaces of the sinuses. The rate of *motion was determined by measuring the time required by* the ink to traverse a distance of 2 mm., and dividing by 2.

THE CILIA AND THE FILM OF MUCUS. The interior of the frontal sinus is, of course, lined by ciliated cuboidal epithelium. It is the coördinated activity of these cilia that furnishes the force that drains the cavity. The epithelium produces a film of mucin that covers the surface. This film is like a secondary membrane that might be called a net of mucin. It is extremely thin, of rather high viscosity, very elastic, and has a certain amount of tensile strength. Knowledge of the characteristics of this net of mucin is essential to understanding of the drainage of the sinus.

The film or net of mucin, pushed along by the cilia beneath, moves in toto in a circular or rather spiral manner. The lines and arrows in figure 1 might give the impression that the mucin flows along only certain lines like a number of parallel streams. This is not so, as the entire film moves at once but it flows much faster along certain lines.

Traction plays a large part in the complete drainage of all the surfaces. There are always some areas in which the ciliary action seems sluggish. By denuding small areas, scars can be produced where there is no ciliary action. The film of mucin slides over such areas by means of traction. The cilia in the adjacent regions drag it along as a net might be dragged by one's fingers. In this way the constant exchange of film of mucin is maintained over all surfaces. Gravity plays only an incidental part. The cilia drag the net upward seemingly as rapidly as downward (fig. 1). The normal film is too thin and too viscid to slide off vertical walls or drip from the under side of the roof. When a quantity of material with considerable depth is present, gravity plays a more important part, and in this particular sinus becomes a handicap.

OBSERVATIONS. All the drops of ink placed within the sinus eventually made their exit through the ostium, but not always by the shortest route. Figure 1 represents a composite map of the movements of the drops of ink in all the sinuses studied. There were variations, but in general the

lines followed were very much as illustrated. Each drop placed in the anterior mesial area described an arc that was a little less than one turn of a rather flat spiral. The lateral arm of the arc of this spiral was generally higher than the mesial. In fact, the directions taken by some drops that stopped on the edge of the wound indicated that the course would normally have been across the under side of the roof had this been intact. In some

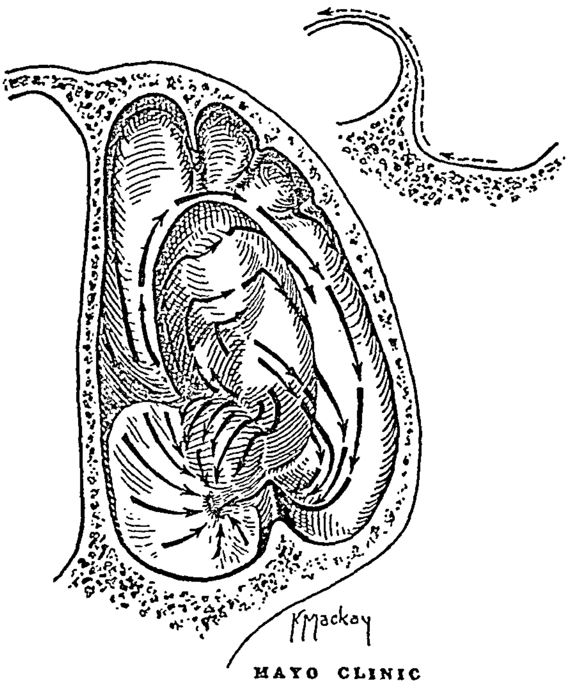

Fig. 1

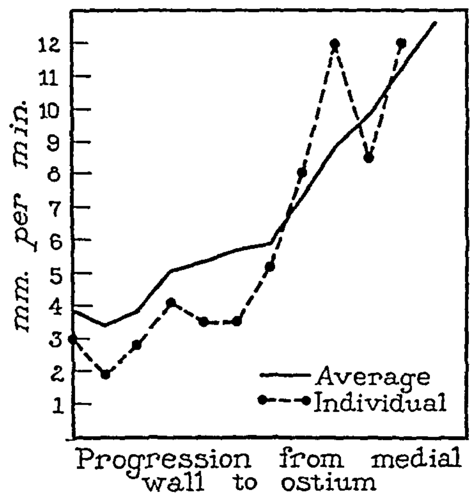

Fig. 2

Fig. 1. Frontal sinus of dog sectioned in a plane parallel with the plane of the frontal bone. The arrows indicate the courses taken by drops of India ink placed at various points on the film of mucin that overlies the normal mucous membrane. Inset: Section in profile, demonstrating abrupt upward slope from deepest part of sinus, around the protruding ethmoid sinus (semicircular cavity in diagram). If the arrows were continued a little farther, they would make egress from the sinus through the ostium. This inset represents a section cut along an imaginary radial line, extending from the lowest point of the sinus to the ostium.

Fig. 2. The increasing speed of drops of ink placed on the mucous membrane in their journey from the anterior mesial wall around the sinus to the ostium may be noted (see fig. 1). The broken line represents a typical single journey, and the solid line represents the averages of thirteen journeys observed in several animals.

of the dogs, the posterior portion of the sinus was elaborately divided by incomplete partitions into a number of deep chambers (fig. 1). When passing over these, the drops of ink, in general, maintained the direction of the individual arcs on which they were traveling; that is, they went deep into the first chamber, around the partition, and deep into the second chamber, then around another partition, and so on (fig. 1). That the dis-

tance across the posterior wall of the sinus might be increased in this manner several times seemed to make no difference. The drainage on the upper surface of the leaf was directed toward the ostium. On the under side, on the other hand, it was directed the opposite way and followed the lines on the mesial wall.

The object of the spiral direction of flow is not entirely clear. It might well be that the purpose is to avoid areas of tension and stasis in the film of mucin so far as possible. In any case it is obvious that there must be some tension somewhere, since there is a difference in direction of flow in those adjacent areas lying near the mesial side of the ostium. In this area, there is a leaf-like structure, covered on both sides with epithelium, that lies against the floor and mesial wall (fig. 1). The thin edge is slightly raised, otherwise the structure is flat. This edge marks the dividing line between the two directions of flow, and seems to divide like a knife-edge the film of mucin that lies over it. This reduces the tension to a minimum. There is a region of tension just posterior and inferior to the edge of this leaf-like structure, where the lines followed by the ink separate in a Y (fig. 1).

Speed-gradient. There was definite acceleration in the rate of travel of drops of ink placed on the mesial wall in their journey to the ostium. The rate just at the approach to the ostium may be from four to six times as great as at the beginning of the spiral on the mesial wall (fig. 2). The acceleration of any individual drop was by no means uniform. It might flow rather rapidly for a time, then hesitate, change direction somewhat, and speed up again.

The reason for this acceleration of speed is obviously to avoid accumulation of mucin at the ostium. All the mucin from the comparatively large area of the surface of the interior of the sinus must pass out through a small opening. If there were no increase in rate at the approach to the outlet, and within the outlet itself, clearly there would be danger of accumulation.

The mechanism by which the acceleration is effected has not been determined. Samples of mucous membrane from various parts of the sinus were removed and placed under the microscope at once, and the rate of ciliary beat was observed. No significant difference in the rate of the beat could be detected in the various samples. Of course, that does not mean that there was no difference in position. It is probable, on the other hand, that there is such a difference, and that the coördinating impulses that bring it about were interrupted when the samples were removed.

Emptying-time. The time required for the sinus to rid itself of various substances and volumes was measured.

When ink was sprayed over all the interior surfaces of the cavity, forming a thin film, a normally active sinus would sweep the major portion

out in fifteen to forty minutes. There were often a few little spots in deep depressions that remained black for an hour or more. This seemed to be due to the fact that a thin, watery secretion, which carried the ink with it, collected in deep depressions after the sinus has been open for a time. It was presumably secreted on account of the unaccustomed exposure.

When a volume of material was dropped into the depth of the sinus, deep below the level of the ostium, the time required for the cilia to move it upward to the ostium depended not only on its volume but on its nature. Thick, heavy mucin was handled best and most rapidly. Pus was not moved so readily, and aqueous solutions required the greatest length of time. The reason is clear. Aqueous solutions can be moved upward

TABLE 1

The time required in twelve tests to move amounts of mucin from the deepest portion of the frontal sinus out through the ostium

TESTS	AMOUNT OF MUCIN	TIME
		minutes
1	1 small drop	11
2	1 large drop	21
3	0.15 cc.	60
4	0.2 cc.	16
5	0.2 cc.	15*
6	0.2 cc.	12
7	0.2 cc.	45
8	0.25 cc.	9
9	0.5 cc.	120
10	1.0 cc.	120
11	1.0 cc. (estimated)	50
12	1.5 cc.	50†

* Two-thirds gone in fifteen minutes. No further progress during next fifty minutes, apparently because of a blood clot in the ostium.

† Almost gone; experiment stopped.

toward the ostium only in extremely thin films, so thin that two or three hours may be required to move even 0.1 cc. On the other hand, 0.25 cc. of thick mucin has been seen to disappear in as little as nine minutes. When this amount of thick mucin was dropped into the sinus, a stringy film promptly began to climb the wall toward the ostium. After a short time it began to exert traction on the remainder, sufficient that the entire globular mass was lifted bodily from the bottom of the cavity and dragged up the wall toward the ostium. Tests were made on seven dogs, using a very thick, mucilaginous, sterile mucin found in certain sinuses that had previously been operated on in connection with some other experiments. This mucin was so thick that it could be picked up from a glass dish by

means of forceps and leave a dry surface. No great uniformity was found in twelve tests made. A volume of 0.5 cc. once required two hours to disappear, whereas, on two occasions, three times that volume required less than an hour (table 1). The anesthetic probably had much to do with this variability. Tests 3 and 6 were made in the same sinus at different times. The rate was much greater in the latter.

Commercial mucin was used in a number of tests without success. Apparently something in the preparation destroyed ciliary action, because no motion could be detected. Pus was used a few times. It moved slowly for a time but motion usually ceased after an hour or two.

Ink diluted with Ringer's solution was used as an aqueous substance. When a volume of this was dropped into the depth of the sinus it was seen to move away steadily with ciliary action, but it required so much time that a watery secretion produced by the mucous membrane due to exposure began to collect in quantities sufficient to maintain the volume. For that reason, it was difficult to measure the emptying time of aqueous solutions unless very small volumes (less than 0.1 cc.) were used. Nevertheless, such materials were moved away completely in the unopened cavity.

SUMMARY AND COMMENT

The drainage of the frontal sinus of a dog was effected by means of the movement of a lining film or web of mucin motivated by ciliary action. This film was extemely thin but of high viscosity and had sufficient tensile strength to sustain a considerable degree of traction. This traction was a very important factor in the drainage. The direction of motion was that of a flat, lateral spiral so arranged that there was but little tension from conflicting currents in the film of mucin. There was a speed-gradient of fairly uniform acceleration (when numerous tests were averaged); the speed at the ostium was four to six times as great as that on the mesial wall. Several different materials were used to test the time required to raise small volumes from the deepest recess of the sinus, upward to the level of the ostium. All were moved more or less successfully, but thick, mucilaginous mucin was moved most completely and rapidly. Roughly, one or two hours were required to move out 1 cc. of thick mucin. A longer time was required to remove creamy pus, and still longer to remove watery substances. It was probable that the rate of drainage was greater and more uniform in the unanesthetized animal with unopened sinuses. These tests were made across a steeply slanting surface. The time required to move these materials was much less when there was no need to lift them against gravity. Here, I am speaking of materials in considerable volume. This concept should not be confused with that offered earlier in the paper; namely, that a thin film moves as fast against gravity as with it. When a thin film of ink, just sufficient to stain the lining of

mucin was sprayed into the cavity, it disappeared almost completely in about thirty minutes, and sometimes in half that time.

Clinical application of these facts can be made readily, if conditions in the sinuses of man are similar, as presumably they are. If bacteria gain access to a sinus and lodge in the film of mucin on the walls, they are swept out in a very short time. If a volume of fluid gains access, or a volume of secretion forms, a much longer time is required to remove it. In general, the more mucin there is in the secretion, the better it drains. Pus drains poorly on account of its physical properties. In addition it may contain toxins or other materials that destroy ciliary action.

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THE EFFECTS UPON CARDIAC MUSCULATURE OF SUB-THRESHOLD ELECTRICAL CURRENTS

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If a cardiac muscle preparation be subjected to stimulation by induction shocks above threshold strength, the preparation may be caused to contract rhythmically up to rates somewhat greater than that of the normal spontaneous rhythm. Faster rates of stimulation may cause irregular responses to appear with many of the applied stimuli ineffective in producing a response. At a rate such as is obtained, for example, by the vibrator on a Porter induction coil, and if the secondary coil be adjusted so that a single break shock is just threshold, continued stimulation will usually cause the preparation to show a single contraction followed by a period of quiescence which lasts until the stimulation is momentarily interrupted. Another period of stimulation will then usually show another single, initial contraction and ensuing quiescence. Using such just threshold shocks, this type of response may often be demonstrated with stimulation rates of only four or five per second. Although this method of stimulation may involve application of an alternating current, unidirectional shocks show the same effect in rather greater degree.

The type of response characterized by a single initial contraction is usually ascribed to an indefinite prolongation by the induction shocks of the relatively refractory phase following the first contraction. However, with still higher rates of stimulation, the shock strengths being somewhat above threshold, there may be contractions both at the beginning and at the termination of the period of stimulation, but none during the continued application of the shocks.

Initial and final twitches were observed in a nerve-muscle preparation by Grunhagen (1872) and Engelmann (1871). Bernstein (1871) had previously described the initial twitch phenomenon. Engelmann later described the appearance of the initial contraction in the bulbus aortae and ventricle of the frog (1878).

The above phenomena appeared to have an underlying relationship with the phenomena described by Wedenski (1883) and Gildemeister (1908). Certain experiments supporting this view were undertaken by one of us several years ago but were inconclusive in consequence of technical difficul-

ties. Recently Erlanger and Blair (1931, a and b) have published results of a study of the effects of electrical currents upon nerve irritability. At an early stage of their experimental work we were struck with the similarity of reaction of the irritable mechanisms of nerve and cardiac muscle. Therefore, provided with an adequate stimulating device, we undertook a study of the changes in cardiac irritability under the influence of electrical currents in a manner which somewhat paralleled the attack which Erlanger and Blair were making upon nerve irritability. Such of our experiments as duplicated theirs in method gave, in most cases, qualitatively identical results. In some respects the results were similar only in general trend and in one respect differed completely. The single case of dissimilarity occurred in that group of experiments in which the effects on irritability of subthreshold and superthreshold stimuli were compared. A striking difference between nerve and heart muscle appeared in consequence of the relatively long contraction time of the heart muscle and the accompanying long period of absolute refractoriness.

The work of Erlanger and Blair, undertaken as an extended investigation of the Gildemeister phenomenon in the light of the work of Kato and his associates (1929), developed into a broader study of the effects upon nerve irritability of 1, subthreshold induction shocks; 2, subthreshold constant currents of various durations; 3, superthreshold constant currents, and 4, currents of strength increasing to above threshold but with a subliminal gradient. Testing irritability with an induction shock and arbitrarily expressing the level of irritability as the reciprocal of shock strength necessary to produce a certain response, Erlanger and Blair found:

1. That during the flow of a subrheobasic current directed cathodally with respect to the region of nerve studied, the curve of irritability, plotted against time, rises at first, attains a maximum level, and then falls back to a lower but still supernormal level at which it remains with only slight changes until shortly before the current is broken.

2. That shortly before the break of a constant current, the irritability curve begins to fall. This "preterminal fall of irritability" is an artifact introduced into the curve because of a period of time which elapses between the application of the induction shock and the beginning of rise of the action potential of the less irritable fibers.

3. That following the break of the cathodal current the irritability persists momentarily above normal, though falling rapidly to and below normal. The time of recovery from this subnormal irritability is similar to that of the duration of the relatively refractory period of the nerve.

4. That after an applied subthreshold induction shock of short duration, there appear effects, qualitatively similar, though not with identical time or intensity relations, to those which occur following the break of a constant current.

5. That the relatively refractory period (measured by induction shock strength necessary to produce a second response) can be nearly abolished by an anodally directed shock applied late in the absolutely, or very early in the relatively refractory period.

6. That following the break of a short anodal current there is a period of decreased irritability followed by one of increased irritability, the time functions of these changes being those of the opposite changes following a short cathodal current.

Certain other phases of their work, some of which we have and some of which we have not duplicated need not be outlined here. The findings of Erlanger and Blair outlined above could be used as a summary for part of our own results. It is in these respects that the irritable mechanisms of nerve and heart muscle show similar behavior. The results of our experiments will be presented and discussed below.

Apparatus and procedure. Non-rhythmic strips were cut from the ventricles of turtles of the genus Pseudemys and were suspended in a moist chamber by the wool wicks of a pair of calomel electrodes. The strips were attached to the wicks with the least possible injury by silk threads which were first tied to the strips, one near either end, and were then tied tightly to the wicks. The series resistance of the pair of electrodes used was approximately 11,000 ohms. A thread attached to the fraenum of the strip was passed through an opening in the top of the moist chamber and over a pulley to a light spring lever which recorded contractions of the strip on a smoked paper. To avoid injury to the stimulated points, which were the points of suspension, the strip was placed under so little tension that there was considerable lost motion in the recording system. The records obtained, therefore, served merely to indicate contraction or lack of contraction (see fig. 1). The more detailed time elements of the mechanical records obtained are of no significance.

For stimulation with two induction shocks, two cored Porter coils were used. The secondaries were connected in parallel to the stimulating electrodes. In most of the experiments a 10,000 ohm resistance was also placed in series between one of the secondary terminals and an electrode. A key operated by the rotating interrupter served to short circuit the make shocks, the break shocks alone being used. Each primary circuit was activated by a 6 volt storage battery connected through a reversing key, thus making possible quick changes in the direction of the secondary current. A variable rheostat in each primary circuit gave sufficient change of current to permit an experiment to be conducted without change of position of the secondary coils. Since the secondary shock strength is nearly proportional to the primary current, the current calibration for different rheostat dial positions gave a relative expression of the strength of the shocks used. A key in each primary circuit was made and broken by the

rotating interrupter. The interrupter was so constructed as to make possible the easy adjustment of the time of opening or closing of any of the three keys with respect to that of the others. The time and sharpness of action of the keys were checked by use of the cathode ray oscillograph. Times of stimulation shown on kymograph records were obtained by signal magnets placed in the primary circuits of the coils.

In using a constant current in conjunction with the shock from a single

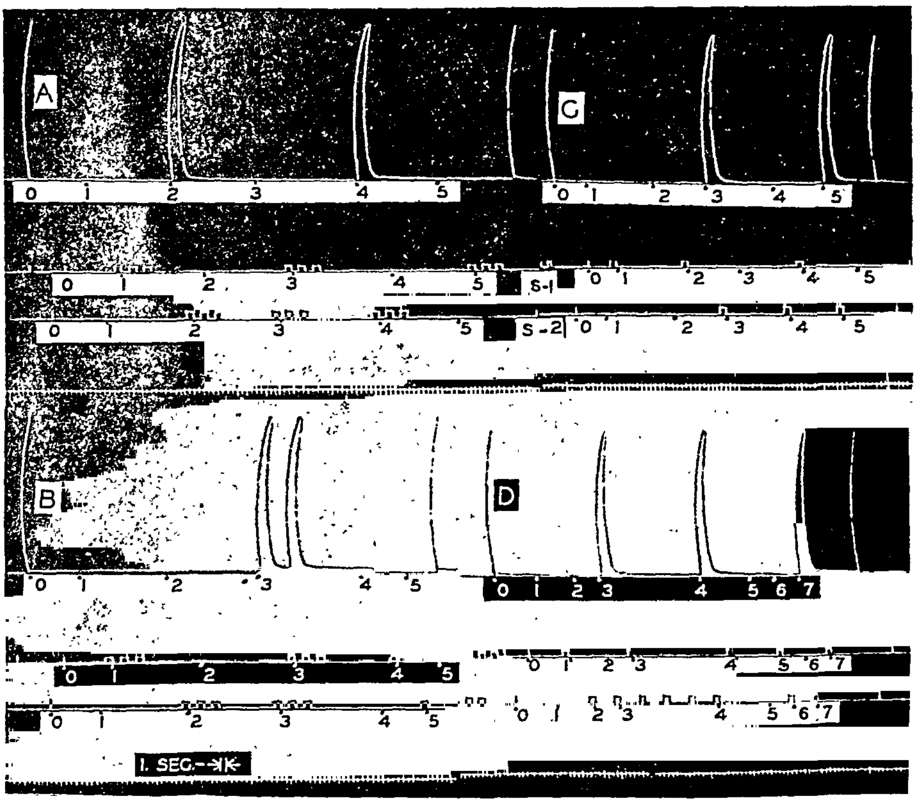

Fig. 1. Records showing enhancement and depression of irritability following subthreshold induction shocks. Shock polarity in all cases stated with respect to point stimulated to give response. Records from top downward in each case are: contraction of strip, first or sensitizing shock, S_1, which is always subthreshold, second or testing shock, S_2, and time intervals in seconds. Numbers on the white strips indicate corresponding times for each record.

A. Depressed irritability following anodal shock. Shock interval 9 sigmas. S_1 anodal. S_2 cathodal, threshold. When S_2 follows S_1 no contraction occurs.

B. Enhanced irritability after cathodal shock. Shock interval 7 sigmas. S_1 cathodal. S_2 cathodal and subthreshold. When S_2 follows S_1 a contraction occurs.

C. Depressed irritability at considerable interval after cathodal shock. Shock interval 54 sigmas. S_1 cathodal. S_2 cathodal, threshold. When S_2 follows S_1, no contraction occurs.

D. Enhanced irritability at considerable interval after anodal shock. Shock interval 54 sigmas. S_1 anodal. S_2 cathodal and subthreshold. When S_2 follows S_1, a contraction occurs.

induction coil, a high resistance (100,000 ohms) voltage divider was used to furnish the desired potential derived from the circuit activated by a 6 volt storage battery. The derived circuit was connected either in series or in parallel with the secondary of the induction coil. With either arrangement a key operated by the rotary interrupter was placed in the battery circuit of the voltage divider so that the shunting of the induction shock might remain unaltered.

The procedure involved in suspending the strips from the electrodes usually resulted in a slightly greater irritability at one end of a strip than at the other. The end showing the greater irritability was, therefore, used for studying thresholds and the current directions are spoken of with respect to this point. In all cases where currents anodal with respect to the end studied were employed, they were checked before and after each group of tests to make certain that stimulation had not occurred at the other end of the strip.

A complicating factor in the determination of threshold by the methods used involved changes in threshold during the course of an experiment. With considerable regularity it was found that a strip when first hung on the electrodes showed an extremely variable irritability. The irritability became stabilized within about 15 minutes and was then maintained in a good preparation for one to three hours, this time depending somewhat on the amount of abuse which the tissue received. Because of the difficulty of obtaining good agreement of threshold values during the early period of changing irritability, it was our custom to attach the strip to the electrodes and then to calibrate our wheel speed, check zero time relations of the keys and make similar adjustments. During this time the strip was occasionally stimulated and the irritability would usually come to a condition which was sufficiently stable to allow thresholds to be determined with an accuracy of from one to five per cent. After a routine experiment had proceeded for some time, the threshold would begin to rise rapidly, and as this rise would continue until the preparation failed to respond, it was customary to terminate the experiment at that time. Experiments were, therefore, done upon strips with a fairly stable and normal condition of irritability. Most of the experiments were done at temperatures of 23°C. to 25°C. The figures given in the diagrams are representative of typical preparations at 25°C.

Experimental results. 1. *Effects of a continued, subthreshold, cathodal, constant current.* Figure 2 reproduces two curves showing the effects upon irritability at the cathode of subthreshold constant currents, curve 1 representing the effects of a stronger current than that used in the experiment which furnished the data for curve 2. The term irritability is used here only to mean the ease with which the tissue responds, by a contraction, to an induction shock. This is arbitrarily expressed in terms of the strength

of a threshold shock, the less the shock strength necessary to give a contraction, the greater the "irritability."

With a continued constant current of relatively long duration (more than 40 sigmas) there is a period of rising irritability of the strip. The curve rises during a period of about 23 sigmas at 25°C. After the crest of the curve is reached there may be a fall which continues fairly rapidly for 20 or 30 sigmas and then tends to flatten out and continue at a more or less constant level. In many apparently excellent preparations the curve rises to the crest and falls off very slightly or not at all as the current continues. In other preparations, using similar current strength, after the drop from the crest, there is a slow continued fall as long as the current is allowed to flow. Such effects are evidently related to the depressing ac-

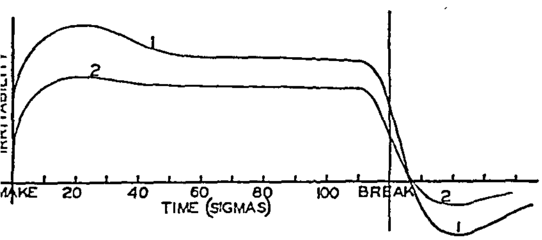

Fig. 2

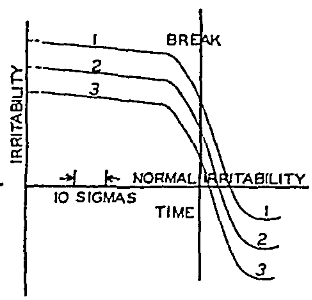

Fig. 3

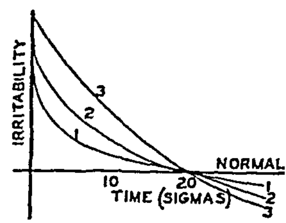

Fig. 4

Fig. 2. Irritability at cathode during and after constant current flow. Curve *1*, current of 60 per cent rheobasic value; curve *2*, current of 40 per cent rheobasic value.

Fig. 3. Effect upon irritability of changes in duration of a current of constant intensity (60 per cent rheobasic). Curve *1*, 60 sigmas duration; curve *2*, 100 sigmas duration; curve *3*, 150 sigmas duration.

Fig. 4. Irritability at cathode following short induction shock. Curves *1*, *2* and *3* representing effects of weak, medium and strong subthreshold sensitizing shocks respectively.

tion of the continued cathodal current and are in part dependent on as yet uncontrolled individual variations in different preparations. It is usually the case that in good preparations subjected to polarizing currents of 40 per cent to 60 per cent threshold strength, the curve reaches its maximum, falls somewhat and then shows a further gradual drop as the current continues. With less strong constant currents, the fall from the crest may be imperceptible. With stronger currents there may be a pronounced and continued fall in irritability to a level even below that of unpolarized tissue.

At a time about 10 sigmas before the break of the current, the curve begins to fall more rapidly, and at the time of the breaking of the current may be at approximately the same level as when the current was made. This level represents simple electrical summation of the stimuli for the condition of the strip at that moment. The "pre-terminal fall," that is, the fall in the curve before the break of the current, is, as was shown by Erlanger and

Blair in nerve, an artifact introduced into the curve by the method of testing and will be discussed in greater detail below.

After the break of the current, the irritability passes toward the line of threshold irritability and crosses it at a time varying, under different conditions, between 3 and 20 sigmas. The greater the drop from crest level at the time the current is broken, the shorter is the time usually required for the curve to reach the base line. This relationship is seen with increasing durations of constant current, longer durations giving a shorter time to base line (fig. 3).

The relative effects of change (1) of strength of a current allowed to flow for a constant time, and (2) of the duration of a current of constant strength, are shown in figures 2 and 3, respectively. It will be seen that in figure 2, curve 2, which was plotted for a 40 per cent threshold polarizing current, shows less enhancement and less depression of irritability than does curve 1, which represents effects of a 60 per cent rheobasic current. The time relationships of the curves are, however, similar. The curves of figure 3 show a quite different state of affairs. It is probable that the beginning of the pre-terminal fall for curve 1 (representing effects of a "short" current flow, 60 sigmas) should be placed slightly earlier than that of curve 3 (long current duration, 150 sigmas). Such a displacement could not be accurately plotted and is omitted from the curves. The time from break of the current to maximum depression is quite constant. The time from the break to the level of normal irritability is, however, much less when a moderately strong current is allowed to flow for a "long" time than when it has acted only for a "short" time. In other words, while the experimental curves show a fairly constant form, the time of enhanced irritability after the break (period of latent addition) depends on the depression of irritability which has occurred during the flow of the current.

The duration of the period of post-cathodal depression is probably quite constant although the intensity of depression at any time depends on the strength of the current used. The irritability recovers along a curve which can easily be plotted for times up to several tenths of a second and which continues an asymptotic approach to the normal irritability level. It has a time function which is similar to and possibly is identical with that of the curve of recovery from relative refractoriness.

With currents which do not produce a considerable depressing effect and in occasional preparations in which there is little or no fall in the curve from crest height during current flow, the post-cathodal depression is reduced to a very small value and may escape observation if it be present at all. Such a curve as that of figure 2, plotted for irritability changes to a prolonged constant current, probably represents as a general case the conditions of which the curves for short constant currents or induction shocks are special cases.

2. *Inhibition of response to a rheobasic constant current of indefinite duration by an anodal shock.* If a rheobasic current of "indefinite" duration be used, there is a utilization period before the tissue responds. That is to say, in order to obtain a response the current must flow for a certain time, the "rheobasic time" (*Hauptnutzzeit*). The response may be prevented from appearing at any time if an anodal shock of proper strength be thrown into the tissue before the response occurs. The curves showing the relations of time and of anodal shock strength necessary to prevent a response were plotted for a number of preparations. In order to prevent the response, a relatively strong shock is required early in the utilization period while a very weak shock suffices just before the end of the utilization period. The time relations of this curve vary slightly in different preparations, but, in general, it may be said that this group of curves has the same time functions as do the curves of rising irritability plotted by testing the effects of a subthreshold constant current by the strength of cathodal induction shocks just necessary to produce a response.

The above method of inhibiting the response to an applied constant current was used to test the nature of the preterminal fall. It seemed probable that this apparent fall in irritability before the breaking of the current might be due either to the fact that the breaking of the current terminated too quickly the flow of what was in reality the stimulating current, or because the anodal effect of breaking the current cut off or blocked a process which had been started by the induction shock but which was subject to a prolonged latency of action. The curves which we obtained showed irregularities of form which we have been unable to explain satisfactorily. Apart from details of curve form, however, these experiments demonstrated conclusively that the preterminal fall represents a delay in response of the polarized tissue after the application of the cathodal induction shock. It was found that the appearance of a response could not be inhibited at a time later than that at which the action potential appeared. This time was the time at which the constant current was broken. We hope to be able to report soon on the extent to which the anodal effect of the breaking cathodal current enters into the determining of the tissue response.

3. *Effects upon irritability of two induction shocks of similar polarity (cathode-cathode series).* *a.* If a slightly subthreshold induction shock be followed within less than a certain critical time interval by a second (also sub-threshold) induction shock, summation may occur and a contraction of the strip be elicited (figure 1). Using a constant strength of first or "sensitizing" shock and recording the strength of the testing shock just necessary to produce a contraction, it is possible to obtain results which may be plotted as in figure 4. As Erlanger and Blair found in nerve, the maximum effect is obtained with simultaneous shocks, the effect becoming less as the interval is increased. The base line, representing normal irri-

tability is reached in the case of heart muscle in about 20 sigmas. A range of from 18 to 23 sigmas covers the results of practically all of our experiments.

b. If a subthreshold sensitizing shock be followed by a second (testing) shock also of subthreshold strength after a time equal to the duration of the summation period (about 20 sigmas), no contraction occurs. In order to elicit a contraction, the testing shock must be brought to threshold strength. This point on the curve represents the transition from the period of enhanced irritability to the period of depressed irritability.

c. If a subthreshold sensitizing shock be followed by the testing shock after a time greater than that of the critical period, a contraction occurs only if the testing shock has a strength greater than the normal threshold. This period of depression can usually be followed for about 0.5 second. However, in occasional preparations we have noted results of previous ineffective shocks which have lasted certainly for a period of three or four times this value and in a few of these cases we have definite figures covering times up to twelve or fifteen seconds. We consider, therefore, that while we are unable to plot accurately the entire course of recovery from post-cathodal depression, its course is similar to that of the recovery from relative refractoriness. The two curves show similar changes and approach the normal asymptotically along a curve which may be traced for several seconds.

4. *Effects of induction shocks of unlike polarity.* *a. Anode-cathode series.* The effects of two shocks of unlike polarity can be adequately presented in brief by saying that the curves of this series are essentially inverted forms of those of the preceding series. For a period of some twenty sigmas after application of an anodal shock, a cathodal shock must be of greater than normal threshold strength in order to elicit a contraction. The curve approaches the base line and crosses it at the same time as do the cathode-cathode curves. There then follows a period during which the effect of the anodal sensitizing shock is to leave the tissue more than normally excitable in response to cathodal shocks. In our experimental procedure, therefore, we routinely determined the time at which the irritability curve crosses the base line by determining the responsiveness of the strip both after anodal and after cathodal sensitizing shocks.

b. Cathode-anode series. If a cathodal shock of threshold strength be followed within a short time by an anodal shock of sufficient strength, a contraction does not occur. The longer the time between the two shocks, the greater must be the strength of the anodally directed second shock if the response is to be prevented from appearing. The maximum time which regularly gave inhibition was eight sigmas. With separation of more than eight sigmas we were able to prevent a response in only one experiment. In that case fifteen sigmas shock separation was used successfully.

We attempted to find a clear cut explanation of the phenomenon by study of cathode ray oscillograph records taken with one lead electrode placed at the point of stimulation. Although we were unable to procure any records in which the shock artifact did not outlast the maximum shock interval for inhibition, comparison of records made by use of sub-threshold and super-threshold shocks failed to reveal any sign of a beginning tissue action potential for a time equal to this maximum shock interval. It appears certain that the second, anodal shock prevents the development of an action potential in the strip by depressing the irritable mechanism *before* the beginning of the action potential as we now know it. By the use of various electrode combinations we have been unable to detect any polarizable system in the stimulating circuit proper which could be considered as responsible for a prolonged current flow. A just threshold induction shock produces a response in turtle ventricular tissue which may not be expressed as an action potential for a period of as much as eight sigmas after the beginning of the shock. This is in amendment to the statement previously published by one of us (Gilson, 1927). An anodally directed shock of sufficient strength thrown into the tissue during this interval may prevent any contraction (or action potential) from appearing.

5. *Effects of continued repetitive stimulation.* If a strip be subjected to repeated stimulation, the threshold gradually increases. This apparently is identical with or related to the phenomenon discussed in a series of papers by Scheminzky and his co-workers (1930). They find that a skeletal muscle, per ex., a frog sartorius, if repeatedly stimulated by brief periods of constant current, by induction shocks, or by condenser shocks gives a mechanical record which appears like that obtained from a muscle showing rapidly developed and severe fatigue. If the direction of the current be reversed, there is immediate and pronounced recovery, followed by a "fatigue" which may again be offset by a reversal of the stimulating current. Especially in working with anodal-cathodal combinations did we find changes in thresholds of the sort which would be expected from Scheminzky's work. Under the conditions of our experiments such effects have, however, been relatively slight. Nevertheless, if a strip be subjected to rather rapid unidirectional stimulation (sometimes as slow as four or five stimuli per second) with an intensity but very little above threshold, there will result the initial contraction followed by a period with no further responses. If after several stimuli have been sent in, an anodal shock be applied at a time such that the next cathodal shock falls during the period of enhanced irritability, there may be a contraction in response to the cathodal shock and then a period of further quiescence. Usually the combination of depression of irritability by the continued cathodal stimulation is so great relatively that continued alternation of anodal and cathodal shocks will not produce a continued series of responses. In this case, as in

the other combinations tried, the variations in actual current flow through the tissue with changes in tissue polarization may be responsible for the changes observed, although this phase of the problem has not been examined.

Using a neon tube stimulator, we investigated the effects of rapid, unidirectional stimulation. It will be seen from the curves presented above that rates of stimulation above 50 per sec. should allow one shock to fall within the summation period left by the preceding shock. Experimentally we found, in accord with previous work, that a strip responds to such stimulation as follows:

a. If the shock strength be barely above threshold there appears the initial contraction only.

b. If the shock be somewhat stronger than this, some preparations, though not all, show an initial cathodal and a final anodal contraction.

c. If the shock strength be still further increased, to three or four times threshold, occasional preparations show rhythmic responses for several beats during stimulation, and then usually a final contraction when stimulation is stopped.

In other words, the effects of rapid unidirectional stimulation approach those of a constant current. The cathodal depression is in most cases so great that, if one uses moderate shock strength, the summation of stimuli which occurs after recovery from the first contraction is still not sufficient to bring about a rise of irritability great enough for the production of a second response. It is probably the polarization involved in this depression which is responsible for the appearance of the final contraction which occurs at the anode when application of the shocks is stopped.

6. *The relatively refractory period and cathodal polarization.* If a strip be made to contract by electrical stimulation and if an anodal shock be applied late in the absolutely or early in the relatively refractory period, the irritability may then be tested by a succeeding cathodal shock. Comparable curves of recovery of irritability may then be plotted with and without the anodal shock. We were unable to effect any change in the duration of the absolutely refractory phase. However, with anodal-cathodal shock separation of 30 to 50 sigmas, we were able to demonstrate an apparent shortening of the relatively refractory phase. Such an interval, however, normally allows for enhancement of irritability to the cathodal testing shock and it is difficult to determine whether the lowering of the threshold toward normal during this time was due to a true shortening of the relatively refractory phase or merely to an unrelated enhancement of the then threshold level as a result of the late, post-anodal enhancement.

Discussion. Our results are compatible with the findings of Keith Lucas on the summation interval in frog heart although he reported merely maximum measurable summation to a 95 per cent threshold shock of only

8 sigmas. It is to be seen that in all of the experiments which we have reported above there is a complete agreement between the work of Erlanger and Blair and our own. Erlanger and Blair meet with the complicating factor that the total response of the nerve trunk subjected to submaximal stimulation is made up of responses from many fibers having different thresholds. Our results are comparable to those which they might have obtained had they been studying a single nerve fiber responding, therefore, in a unitary manner.

The striking difference between their work and our own is that centering about the fact that heart muscle contracts and a nerve does not. Experiments on frog nerve at 25°C. yield curves of irritability changes in consequence of subthreshold currents which may be made generally representative of those for turtle ventricular muscle by multiplying all time values by about 40. However, if the two tissues be made to respond and the irritability changes in the relatively refractory period then be studied, the nerve passes quickly through its absolutely refractory phase and becomes relatively refractory in some 0.7 sigma, whereas, the heart strip is absolutely refractory for perhaps 0.7 second. Shocks applied to ventricular strips early in the systolic phase, therefore, have little if any effect on the course of the relatively refractory phase. In nerve, a subthreshold shock causes changes in irritability which allow the nerve to be permanently restored to normal levels in about the same time that would be required had a superthreshold shock been used. In heart, on the other hand, a subthreshold shock causes enhanced irritability for perhaps twenty sigmas, and demonstrable depressed irritability for several tenths of a second, with recovery to perhaps five per cent of normal in half a second. If we assume that the curve is that of the relatively refractory phase, we may say that the recovery from relative refractoriness begins, at most, about fifty sigmas after application of the subthreshold shock. However, if the shock be of more than threshold value, there is interpolated between the stimulation phenomena and the recovery phase an absolutely refractory period of several tenths of a second. It would appear, therefore, that the typical tissue reaction, occurring in response to some threshold trigger mechanism holds in abeyance the recovery of the irritable mechanism which would have occurred along the same curve but at an earlier time if threshold value had not been reached.

Excepting this one striking difference between nerve and muscle, the similarity of reaction is remarkable. Moreover the close agreement of the nerve and heart experiments serves as an indication of the validity of both and with the material of both researches available for comparison, certain generalizations may be made. It will be noticed that the times 1, from the make of the current to the crest of the irritability curves of figure 2; 2, from the break of the current to maximum depression of figure 2, and 3

of maximum summation interval for two cathodal induction shocks (fig. 4) all show similar values of about 20 sigmas. This value is also the maximum reached as a limit for the summation interval after the break of a weak constant current which produces no measurable post-cathodal depression. Examination of the figures published by Erlanger and Blair reveals a corresponding similarity of these time values for nerve (Erlanger and Blair, 1931a, fig. 4; 1931b, figs. 2 and 9). It seems, therefore, not improbable that the primary mechanism being dealt with is the same in each case. We cannot at present describe the nature of this mechanism, although time relations such as those revealed by experiment would hold, as would also the relationship between time or current strength and the intensity of the post-cathodal depression, if the mechanism were a polarizable system acted on by the externally applied voltage (cf. Bishop, 1928).

A second striking similarity of reaction in heart and nerve is the apparent latency to electrical stimulation, either to a brief induction shock alone or to an induction shock applied during a period of constant applied voltage. Both heart and nerve show similar "preterminal fall" phenomena. Moreover the experiment of Erlanger and Blair in which they short-circuited their stimulating induction coil at a time such that there was a measurable interval before the beginning of the nerve response seems to indicate that by their induction shock they had started a process which required a considerable time for completion. Upon completion of the process the action potential appeared and propagation of an impulse was commenced. In both heart and nerve, the limiting time value of this secondary process, if it exist, is shortened as shock strength is raised above threshold. Further discussion of the problem will be reserved until further data on experimental material is available.

SUMMARY

Turtle cardiac muscle has been studied with respect to its changes in irritability during and following the application of various subthreshold currents.

The changes found are essentially the same as those described for nerve if the time values for the latter be multiplied by a factor of about 40. The possible implications of the experimental findings in the light of other work are briefly considered. The experimental findings offer explanation of the phenomena of initial and final contractions observed in cardiac muscle under the effect of continued constant current or repeated induction shocks of threshold or slightly superthreshold intensity.

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THE COMPARATIVE EFFECTS ON CARBOHYDRATE METABOLISM OF EXHAUSTING MOTIVE AND EMOTIVE RESPONSES AND EXPOSURE TO COLD

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In the course of a series of investigations on changes in carbohydrate metabolism under different conditions (Britton and Silvette, 1931, 1932) it became necessary to inquire into the effects normally produced by muscular and emotional reactions in a number of control observations. Vigorous cats and kittens, which were used in the reported experiments, were found to be readily responsive subjects in such an investigation. It was soon observed, however, that the slighter motive and emotive responses which were evoked on merely handling the animals, and in withdrawing blood samples, resulted in relatively small blood and extravascular carbohydrate changes. In order to establish reactions which would be as conclusive as possible it was decided to set up fairly exacting conditions, and prolonged emotional and muscular activities to the point of exhaustion were therefore imposed on the animals used in the present study.

METHODS. The experiments were divided into three groups, in addition to the control series. Analyses were made to determine the muscle and liver glycogen, and the blood sugar and lactic acid on each animal. Normal adult cats, and also kittens weighing between 0.5 and 1 kilo (2 to 4 months old) were used in most of the experiments. In the investigations on cold exposure a number of marmots (*Arctomys monax*) and opossums (*Didelphys virginiana*) were also used. In two control series 11 normal adult cats and 9 normal kittens were fasted 24 hours and 18 hours respectively.

Small cats or kittens were placed in a bath of agreeably warm water and allowed to carry out active swimming movements. After the initial wetting the experience was apparently not discomforting. When fatigue supervened, they were removed and sacrificed and the tissues analyzed.

The effects of emotional excitement on carbohydrate levels were tested

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by putting vigorous cats in a small cage and bringing before them an aggressive, barking dog. The imprisoned animals reacted by snarling, showing the teeth, and occasionally striking out with claws bared. At the end of five minutes the cats were removed from the cage and immediately used as stated above.

In the third series of experiments young cats were first subjected to complete wetting of the fur, and then placed in a cold room at a temperature between 0–5°C. Shivering movements occurred soon after exposure in most cases, and signs of muscular stiffness were observed from one to three hours later. The animals were then removed and sacrificed for tissue analysis. Similar observations were made on groundhogs and opossums. Deep rectal temperatures were taken in all cases.

The routine followed in sacrificing and taking samples from each animal was in all cases identical in order to secure comparative results. The animal was stunned, the abdomen and thorax quickly opened along the midline, and the blood drawn directly from the heart into a small amount of powdered sodium oxalate. A portion of the liver was clipped off and plunged immediately into sodium hydroxide solution, and the central portion of the hamstring muscles of the right leg was similarly treated. The whole procedure took somewhat less than two minutes, from the time of stunning until the tissues were immersed in sodium hydroxide.

Blood sugar was determined by the method of Folin and Malmros (1929); the glycogen by a modification of Pflüger's method suitable for amounts of tissue from 0.5 to 1 gram (Bollman, Mann and Magath, 1925), as follows:

Centrifuge tubes containing 2 cc. of 20 per cent sodium hydroxide were weighed, approximately 1 gram of tissue placed in each, and the tubes weighed again. The tubes were immediately plunged into boiling water and heated with occasional shaking for 4 hours. Then 2 cc. of distilled water were added to each tube followed by 8 to 9 cc. of 95 per cent ethyl alcohol. The contents were thoroughly mixed and the tubes allowed to stand over night for the glycogen to precipitate. The tubes were then centrifuged, the alkaline alcohol decanted off, and the impure glycogen washed successively with 66 per cent alcohol (twice), 95 per cent alcohol, absolute alcohol, and ether. The dry glycogen was dissolved in boiling water, 1 cc. of concentrated hydrochloric acid added, and the mixture hydrolyzed in a boiling water bath for three hours. The hydrolysate was then cooled, neutralized with sodium hydroxide, and made up to volume. Glucose determinations were then made on aliquot parts.

Lactic acid was determined by the method of Friedemann, Cotonio and Shaffer (1927), following removal of the glucose from the Folin-Wu filtrate by the copper sulphate-calcium hydroxide method of Van Slyke (1917).

A series of lactic acid determinations, using standard solutions of zinc d-lactate, gave an average recovery of 98.9 per cent over a range of concentrations from 9 to 180 mgm. per 100 cc. (table 1).

Results. The results of analyses for muscle and liver glycogen, blood

sugar, and lactic acid on normal adult cats are given in table 2, and o normal kittens in table 3. It should be noted that the muscle glycogen

TABLE 1

Showing the recovery of lactic acid from various concentrations of zinc d-lactate solution

Average figures

0.001 N ZINC D-LACTATE	0.002 I_2, CALCULATED EQUIVALENT	0.002 I_2 USED	0.002 I_2 LESS BLANK	PER CENT YIELD
cc.	*cc.*	*cc.*	*cc.*	
0	0	0.25		
2	2	2.30	2.05	102.5
4	4	4.20	3.95	98.8
6	6	6.20	5.95	99.2
8	8	7.65	7.40	92.5
10	10	10.40	10.15	101.5
Average....................				98.9

TABLE 2

Glycogen, glucose and lactic acid values in normal adult cats

CAT NUMBER	DATE	MUSCLE GLYCOGEN	LIVER GLYCOGEN	BLOOD SUGAR	BLOOD LACTIC ACID
		per cent	*per cent*	*mgm. per cent*	*mgm. per cent*
10	10/ 7/31	0.374	1.51	85	34
11	10/ 7/31	0.538	1.28	74	26
12	10/ 7/31	0.442	1.42	80	26
16	10/13/31	0.466	0.95	95	25
17	10/13/31	0.404	1.57	78	18
50	12/ 4/31	0.512	0.67	106	23
51	12/ 4/31	0.405	1.43	98	30
54	12/11/31	0.397	0.97	118	17
55	12/11/31	0.412	1.25		
56	12/12/31	0.337	1.52	90	23
57	12/12/31	0.395	0.86	96	23

TABLE 3

Glycogen, glucose and lactic acid levels in normal young cats

CAT NUMBER	DATE	WEIGHT	MUSCLE GLYCOGEN	LIVER GLYCOGEN	BLOOD SUGAR	BLOOD LACTIC ACID
		kilos	*per cent*	*per cent*	*mgm. per cent*	*mgm. per cent*
28	10/22/31	0.55		1.79	99	43
29	10/22/31	0.59	0.208	1.49	111	38
30	10/23/31	0.82	0.294	1.26	91	38
31	10/23/31	0.52	0.287	0.80	81	41
32	10/27/31	0.50	0.269	3.56	112	39
33	10/27/31	0.41	0.268	2.86	110	35
34	10/27/31	0.34	0.228	1.12	93	30
58	1/ 6/32	0.63	0.241	2.72	77	
59	1/ 6/32	0.64	0.420	1.48	68	

TABLE 4

Carbohydrate changes in young cats which were exercised to exhaustion

CAT NUMBER	DATE	WEIGHT	MUSCLE GLYCOGEN	LIVER GLYCOGEN	BLOOD SUGAR	BLOOD LACTIC ACID
		kilos	*per cent*	*per cent*	*mgm. per cent*	*mgm. per cent*
35	11/ 2/31	0.42	0.202	0.426	142	86
36	11/ 2/31	0.76	0.209	0.231	132	90
37	11/ 2/31	0.57	0.156	0.170	160	74
38	11/ 4/31	0.64	0.165	0.221	145	107
39	11/ 4/31	0.70	0.103	0.151	125	77
40	11/ 4/31	0.56	0.201	0.228	156	91
60	2/15/32	0.43	0.203	0.314	132	

levels of kittens are lower than those found in adult cats fasted for a similar period of time, while the blood lactic acid figure is about 50 per cent

higher. Kittens are much more susceptible to the influence of a short fasting period; in part, perhaps, because they are comparatively more active than adults, and thus would utilize their fuel reserves faster.

After muscular exercise (table 4) the muscle glycogen is reduced about 50 per cent, and the liver glycogen to about one-fifth of the normal value, while blood-glucose and lactic acid levels are raised respectively about 50 and 200 per cent. It should be mentioned that the animals were subjected to a brief period of exhausting exercise in which the factor of muscular activity was attended to a slight extent by the emotional state.

Adult cats which were subjected to profound emotional excitation by a barking dog, attended by very little muscular activity, showed fluctuations in carbohydrate values similar to those found in young cats which were vigorously exercised: muscle and liver glycogen were much reduced, and the blood sugar and lactic acid percentages were increased (table 5).

TABLE 5

Carbohydrate changes in cats emotionally excited by barking dog

CAT NUMBES	DATE	MUSCLE GLYCOGEN	LIVER GLYCOGEN	BLOOD SUGAR	BLOOD LACTIC ACID
		per cent	*per cent*	*mgm. per cent*	*mgm. per cent*
13	10/ 7/31	0.168		190	58
14	10/ 7/31	0.239	0.427	196	86
15	10/ 7/31	0.265	0.623	189	50
18	10/13/31			174	77
19	10/13/31	0.131	0.232	175	85
20	10/13/31	0.190		158	41
21	10/15/31	0.260	0.594	94	45
22	10/15/31	0.181	0.596	91	48
61	2/15/32	0.189	0.532	164	
62	2/15/32	0.321	0.682	178	

TABLE 6

Carbohydrate changes in young cats exposed to cold

CAT NUMBER	DATE	WEIGHT	MUSCLE GLYCOGEN	LIVER GLYCOGEN	BLOOD SUGAR	BLOOD LACTIC ACID
		kilos	*per cent*	*per cent*	*mgm. per cent*	*mgm. per cent*
41	11/19/31	0.58	0.175	0.131	49	23
45	11/23/31	0.68	0.137	0.172	61	21
46	11/23/31	0.87	0.168	0.370	69	28
47	12/ 4/31	0.79	0.122	0.326	68	37
48	12/ 4/31	0.57	0.147	0.437	62	24
49	12/ 4/31	1.14	0.136	0.166	56	40
63	2/18/32	1.05	0.224	0.166		
64	2/18/32	0.77	0.260	0.443		
65	2/18/32	0.91	0.198	0.267		

Kittens subjected to wetting and exposure to the temperature of the cold room showed decreases in muscle and liver glycogen which were very similar to those found in kittens after exercise. The blood lactates, however, were but slightly changed from the normal level, although the blood sugar fell in accord with the falling body temperature of the experimental animals (table 6).

In a series of groundhogs which were amytalized and then subjected to a 10-hour exposure to zero temperatures, it was found that the muscle and liver glycogen showed 50 per cent reductions from the normal. The

blood-glucose readings were diminished by about 25 per cent, while the blood lactic acid did not change significantly.

Normal opossums which were previously placed in a cold room for a week, and then sacrificed for tissue and blood analysis, showed in comparison to a control (unexposed) series only a slight reduction in the amount of liver glycogen. The muscle glycogen, blood sugar and lactic acid values did not change appreciably after exposure to cold.

The slight excitement and struggling incident to the preliminary withdrawal of blood from the peripheral vessels of the pinna of cats were found to have only a minor influence on the concentration of blood sugar and lactic acid. Blood samples taken immediately before and after stunning an animal showed, moreover, very little variation in content: the blood sugar rose a few milligrams and the lactic acid only slightly.

Samples of blood taken from kittens which were made to struggle vigorously for a minute or two showed, however, that after excitation the blood sugar had increased about 50 per cent over the resting value, while the blood lactic acid had risen from 26 to 61 mgm. per cent. Ten minutes after exercise the levels had fallen to slightly above normal, and half an hour afterwards the normal levels were again reached. Brief protocols are given below:

Cat 26. October 20, 1931, fasting blood sugar, 93 mgm. per cent; lactic acid, 28 mgm. Immediately after 1 minute of struggling, B.S. 147 mgm., L.A. 75 mgm.; 10 minutes after, B.S. 128 mgm., L.A. 36 mgm.; 30 minutes after, B.S. 70 mgm., L.A. 32 mgm.

Cat 27. October 20, 1931, fasting blood sugar, 95 mgm. per cent; lactic acid, 25 mgm. Immediately after 1½ minutes of struggling, B.S. 118 mgm., L.A. 46 mgm. 10 minutes after, B.S. 79 mgm., L.A. 32 mgm.; 30 minutes after, B.S. 81 mgm., L.A. 24 mgm.

Our results on the effect of muscular exercise on carbohydrate levels are in general in keeping with those reported by earlier workers, who have used chiefly excised tissues or operated animals. In only a few cases have liver, muscle and blood changes in the whole organism such as we have considered been investigated. The recent reports of Long (1928) and Long and Grant (1930) contain references to the earlier work. The McGill investigators used electrical stimulation in their studies on the rat, and found that liver and muscle glycogen and also the blood sugar values were decreased after artificially inducing muscular activity.

In the case of vigorous but brief periods of muscular exercise in our studies, the glycemic levels have invariably been found to be augmented. Dogs which we have run for one or two hours in a treadmill do show, nevertheless, considerably reduced sugar values (Eagle and Britton, 1932). The latter observations are in line with many which have been made on marathon and other runners. The present observations on changes which

are effected by emotional responses are supplementary to some earlier reports (Cannon and Britton, 1927; Britton, 1928; Britton, Geiling and

TABLE 7

Effects on various animals of exposure to cold

ANIMAL NUMBER	WEIGHT	BLOOD SUGAR CHANGES	RECTAL TEMPERATURE CHANGES	HEART RATE CHANGES	REMARKS
			Cats		
	kilos	*mgm. per cent*	°C.	*beats per minute*	
1	1.60	73– 85	39.3–38.0	270–240	Recovered
2	0.76	82– 38	38.3–30.5	180– 64	Died
3	0.98	76– 41	38.4–35.5	192–162	Recovered
4	0.75	62– 48	38.3–22.5	188– 52	Recovered
			Groundhogs		
1-A	2.15	148– 63	38.4–35.9	260–104	Recovered
2-A	1.70	78– 29	35.1–30.0	210– 30	Died
3-A	0.87	160– 32	36.5–17.3	210– 22	Died
4-A	3.13	125– 69	36.4–27.5	240–104	Recovered
5-A	2.47	134–154	38.8–36.5	140–110	Recovered
6-A	1.93	160–167	38.9–36.5	270–160	Recovered
7-A	2.87	174–129	36.8–36.6	228–180	Recovered
			Opossums		
1-B	1.69	96– 69	34.8–32.2	130– 80	Recovered
2-B	0.74	106– 70	36.3–36.0	210–140	Recovered
3-B	0.72	67– 38	33.0–13.8	240– 10	Died

TABLE 8

Average carbohydrate levels in animals under various experimental conditions

TABLE NUMBER	NUMBER OF CATS USED	EXPERIMENTAL CONDITIONS	MUSCLE GLYCOGEN	LIVER GLYCOGEN	BLOOD SUGAR	BLOOD LACTIC ACID
			per cent	*per cent*	*mgm. per cent*	*mgm. per cent*
2	11	Normal adult cats, fasted 24 hours	0.426	1.22	92	25
3	9	Normal young cats (kittens) fasting 18 hours	0.277	1.90	94	38
4	7	Young cats exercised to exhaustion	0.177	0.249	142	88
5	10	Cats emotionally excited by barking dog	0.216	0.528	161	61
6	9	Young cats exposed to cold	0.174	0.275	61	29

Calvery, 1928). The comprehensive studies of the Harvard school (Cannon, 1929) demand no elaboration here.

It is pertinent to emphasize that the influences of emotion on important chemical constituents of the body are essentially similar to those which are brought about by severe muscular exertion. Both motion and emotion result in release to the blood stream and degradation in the tissues of energy-supplying substances, and concurrent accumulation of the products of tissue oxidation or metabolites.

In the cooled animal the changes are again similar; except that when severe conditions are imposed, or when the animal is apparently overcome by the exposure, the blood glucose levels are observed to be greatly depressed, while the lactates do not accumulate and are perhaps more economically synthesized (table 7).

The foregoing studies present concretely the carbohydrate changes which take place when intact, unoperated animals are subjected to various but common experimental exigencies (see table 8). They re-emphasize the great lability and yet profound importance of carbohydrate values in relation to the immediate well-being of the organism.

SUMMARY

The effects of various exhausting conditions on the carbohydrate values in normal cats are considered.

When short periods of vigorous muscular exercise such as swimming are undertaken until an animal is fatigued, the muscle and liver stores of glycogen suffer depletion, and the blood glucose and lactates are concurrently augmented.

Profound emotional excitation for a brief period also brings about changes in carbohydrate values essentially similar to those observed after severe exercise.

On exposure of animals to cold to the point of inducing slight narcosis, reductions commonly occur in blood glucose and lactic acid as well as in the liver and muscle glycogen levels. In such cases the body temperature and heart rate also are reduced. Maintenance of the blood sugar level is invariably associated with maintenance of the body temperature within normal limits.

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EFFECTS OF CORTICO-ADRENAL EXTRACT ON CARBOHYDRATE METABOLISM IN NORMAL ANIMALS

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In earlier reports attention has been drawn to the wide influence on the organism of the newly-developed hormone-containing extracts of the adrenal cortex. The low blood sugar values which occur in adrenalectomized animals are raised, and the high blood cell volume, non-protein nitrogen and lactic acid levels are lowered when the extract is injected. The reduced body temperature and pulse readings are concurrently brought within normal limits. Glycogen in the liver and in muscle, found in adrenal insufficiency to be greatly depleted, is also fully restored (Britton and Silvette, 1931, 1932).

Many of these changes occur also in normal animals. The running ability and energy output of unoperated dogs may be greatly increased for a period of several days after injection of the extract (Eagle and Britton, 1932). Induction of precocious sexual maturity in animals (probably by the action of a second hormone which appears to be contained in our cortical extracts) is also of considerable significance (Corey and Britton, 1931, 1932). Support of the latter observation, it may be mentioned, has already been presented (Atwell, 1932).

Reduction of the high levels of serum calcium and phosphorus found in animals with adrenal insufficiency has also been noted. Variations in blood cell counts in animals have been observed to follow injection of the material. Furthermore, glycolytic effects may apparently be produced by the extract *in vitro*. In various affections in man the blood glucose and arterial pressure have been increased, and the blood non-protein nitrogen diminished, while the working ability has been markedly improved. (Unpublished results.)

These and other related effects have been established in recent studies

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on cortico-adrenal extract made according to a slightly modified Swingle-Pfiffner method in this laboratory. Some reports by other investigators on the influence of the extract, particularly on metabolism, have also recently appeared. The profound influence of the preparation on the organism is thus becoming more and more apparent.

In the present investigation earlier leads have been followed in an attempt to establish the principal effects of the extract. The influence of cortico-adrenal secretion on bodily economy would in this way, it was considered, also be indicated. Our observations have lately been concentrated on the effects on carbohydrate metabolism, for many reasons advanced in previous reports, and particularly because of the experimental results we have obtained in the past few years (Britton, 1930; Britton and Silvette, 1931, 1932).

Normal young male and female rats have been used chiefly in the following series of experiments. Animals weighing between 30 and 60 grams were found most responsive to the injections, and their use greatly conserved the (still highly-expensive) extract. In many of the experimental groups whole litters were used. Numerous experiments were also carried out on adult rats and cats. When large doses of extract were employed, changes appeared in these animals similar to those in young individuals, as will be evident hereafter. A short series of rabbits (nine) has also been used by way of comparison.

All rats were fed alike on a mixed diet. Tests were made after different fasting periods (see tables), when water was supplied *ad lib.*; in one series of controls, examination of the tissues of fully-fed animals was carried out.

The extract was made up in the concentration of 1 cc. per 40 grams of fresh whole adrenal glands. Many different batches of extract, proven to be effective in abolishing the symptoms of adrenal insufficiency but varying somewhat in potency, were used. Usually the material contained about 1:2,000,000 adrenalin as tested by the surviving-intestine method. In most of our controls, which were performed in each series along with the principal experiments, we have therefore employed adrenalin in this concentration, although in some cases we have also tested stronger solutions. Saline controls were also carried out. All injections were made intraperitoneally according to body weight.

In several series of experiments glucose was injected with the extract, to determine the effect on glycogen synthesis. Adrenalin and saline-glucose controls were run simultaneously. This phase of our investigation is being continued.

The blood sugar method of Folin and Malmros (1929) was used. Glycogen was determined according to a modification of the Pflüger technique (Silvette and Britton, 1932). Very careful routine procedures were adopted in taking all tissue samples. Lactic acid determinations were

made in some of the earlier experiments, but the changes in normal animals did not appear significant.

Results are given in the accompanying tables. In the interests of space economy, dates, weights, sex and other dispensable data are omitted. It will be noted that in the earlier experiments large (total) amounts of extract were employed; later it was found that much smaller quantities were effective. Different time periods and amounts of extract were considered, in order to ascertain the conditions necessary to bring about an optimum response. The possibility that the action of the extract on carbohydrate

TABLE 1

Carbohydrate levels in normal young rats, 30–60 grams in weight, treated with cortico-adrenal extract (Group A), or adrenalin in equal concentration (1:2,000,000; Group B), or normal saline solution (Group C)

Food removed and six hourly injections, each 1 cc. per 25 grams weight, given intraperitoneally. Animals sacrificed 1 hour after sixth injection.

RAT NUMBER	MUSCLE GLYCOGEN	LIVER GLYCOGEN	BLOOD SUGAR	RAT NUMBER	MUSCLE GLYCOGEN	LIVER GLYCOGEN	BLOOD SUGAR
Group A: Extract treated				Group B: Adrenalin controls			
	per cent	*per cent*	*mgm. per cent*		*per cent*	*per cent*	*mgm. per cent*
30	0.312	0.840	114	33	0.131	0.261	115
31	0.282	0.955	168	34	0.151	0.222	85
32	0.686	0.823		35	0.114	0.122	101
36	0.515	0.769	122	39	0.286	0.349	93
37	0.463	0.788	132	40	0.276	0.392	112
38	0.484	0.945	165	41	0.341	0.410	124
44	0.430	0.632	138				
45	0.468	0.647	126	Group C: Saline controls			
46	0.530	0.723	116				
47	0.458	0.663	111	28	0.180	0.154	82
				29	0.273	0.492	86
				42	0.247	0.407	68
				43	0.250	0.329	77

levels in the intact animal might be utilized for biological assay of the material was also kept in mind.

Table 1 reveals that the blood glucose and liver and muscle glycogen values are considerably higher in young extract-treated rats than in the adrenalin or saline-injected controls. The animals were given six hourly injections.

Slightly larger hourly doses of extract brought about still higher glycogen and sugar levels (table 2, group A). When other animals were examined at different time-periods after the injection of a single dose of extract, however, the increments were found to be almost equally great

(table 2, group B). In these experiments blood sugar and muscle glycogen levels appeared to be rather rapidly augmented, and were highest at three hours after extract injection, while liver glycogen values reached a peak somewhat later—at six hours after extract was given.

Consistently enhanced glucose and glycogen readings were also obtained when smaller single injections of extract were administered (table 3, group A). The use of increasingly large doses in different animals brought

TABLE 2

Blood glucose and liver and muscle glycogen levels in unoperated rats under various conditions

Groups A1, A2, A3: Adults, 100–150 grams weight, injected with cortico-adrenal extract or adrenalin or saline solution, 5 cc. per 100 grams weight per hour for 6 hours; food removed before first injection; animals killed 1 hour after sixth injection.

Groups B1, B2, B3, B4: Young rats, 30–60 grams weight, fasted 12 hours, then given 1.5 cc. extract per 25 grams weight; tissues taken at end of 1½, 3, 4½ and 6 hours respectively.

RAT NUMBER	MUSCLE GLYCOGEN	LIVER GLYCOGEN	BLOOD SUGAR
Group A1: Extract treated			
	per cent	*per cent*	*mgm. per cent*
51	0.498	1.00	
52	0.523	1.12	435
53	0.553	1.32	200
Group A2: Adrenalin controls			
54	0.136	0.38	124
55	0.234	0.29	131
56		0.46	111
Group A3: Saline controls			
127	0.370	0.34	103
128	0.413	0.27	99
Group B1: Extract treated			
	per cent	*per cent*	*mgm. per cent*
57	0.398	0.782	114
58	0.410	0.626	105
Group B2: Extract treated			
59	0.530	0.816	123
60	0.502	0.923	114
Group B3: Extract treated			
61	0.498	0.869	118
62	0.433	0.841	98
Group B4: Extract treated			
63	0.399	0.956	107
64	0.479	0.887	105

about notably higher blood sugar levels, with no greater accompanying augmentation in the glycogen percentages (table 3, group B). It will again be observed that in the adrenalin and saline controls the values lagged far behind those in the extract experiments.

Throughout the different series of experiments the glucose and glycogen levels after extract injection do not vary considerably, except that after the larger dosages somewhat higher values are attained. The extract-

treated cases invariably showed much higher readings than the controls. The results in the two types of control experiments do not differ greatly,

TABLE 3

Carbohydrate values in normal young rats, 50–60 grams weight, under various conditions

Groups A1, A2, A3: Three hours after food removed, animals injected with extract, adrenalin and saline solution, 1 cc. per 25 grams weight; sacrificed 3 hours later.

Groups B1, B2, B3: Fasted 4 hours, injected with different amount of extract, or adrenalin or saline; tissues taken one hour after injections given.

RAT NUMBER	MUSCLE GLYCOGEN	LIVER GLYCOGEN	BLOOD SUGAR	RAT NUMBER	AMOUNT MATERIAL GIVEN	MUSCLE GLYCOGEN	LIVER GLYCOGEN	BLOOD SUGAR
Group A1: Extract treated				Group B1: Extract treated				
	per cent	*per cent*	*mgm. per cent*		*cc. per 100 gms. body weight*	*per cent*	*per cent*	*mgm. per cent*
98	0.590	1.02	106	106	4	0.450	1.12	141
99	0.530	1.00	113	107	4	0.430	1.41	120
100		0.82	110	108	12	0.407	1.29	210
101	0.660	0.99	114	109	12	0.570	1.36	182
102	0.550	1.12	123	110	16	0.552	1.38	197
				111	16	0.500	0.95	181
Group A2: Adrenalin controls				Group B2: Adrenalin controls				
103	0.369	0.23	86					
104	0.295	0.26	90	112	12	0.268	0.29	141
105	0.341	0.31	79	113	12	0.266	0.31	151
Group A3: Saline controls				Group B3: Saline controls				
125	0.310	0.21	82	123	12	0.325	0.36	91
126	0.284	0.21	86	124	12	0.315	0.43	94

TABLE 4

Blood glucose and liver and muscle glycogen levels in normal (uninjected) fully-fed rats

RAT NUMBER	MUSCLE GLYCOGEN	LIVER GLYCOGEN	BLOOD SUGAR
	per cent	*per cent*	*mgm. per cent*
130	0.487	1.20	113
131	0.501	1.08	124
132	0.496	1.08	102
133	0.468	1.04	106
134	0.363	0.73	105
135	0.483	1.18	128

except that in the adrenalin-injected cases slightly higher blood sugar and lower muscle glycogen values were observed. The average figures covering all the experiments performed are given in table 6 (see also figure 1).

The effects of the extract on normal young rabbits (see tables 5 and 6) and on cats were essentially similar to those observed on rats.

The changes in liver glycogen would appear to us at the present time to give the most emphatic indication of the potency of cortico-adrenal extracts. When injected intraperitoneally into three-hour fasting young rats of approximately 50 grams in weight, 1 cc. of extract (equivalent to 40 grams of whole adrenal glands) per 25 grams body weight should raise the liver glycogen approximately to 1 per cent within a period of two hours. This represents the simplest biological test of the material we

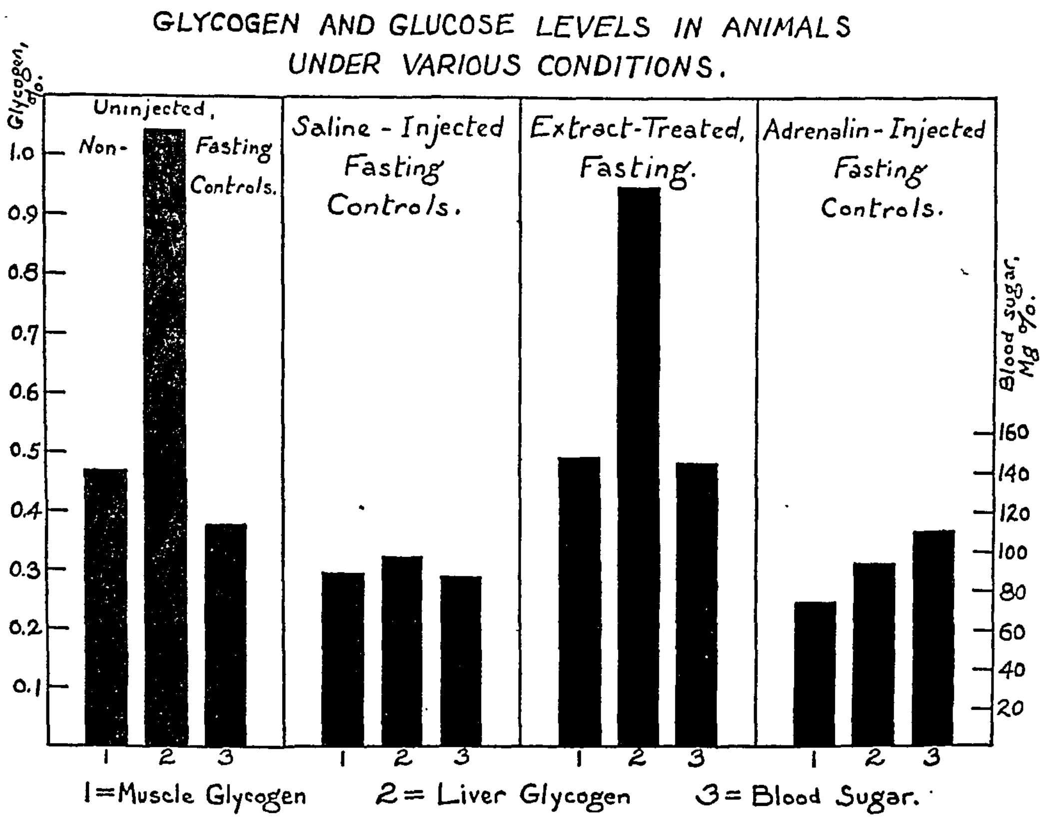

Fig. 1

have devised, although further tests on excised tissues which are now in progress offer considerable promise. It appears superior and far more economical of time and extract than tests involving restoration of adrenalectomized animals suffering with symptoms of insufficiency.

The origin of the increased amounts of glucose and glycogen which are found in the tissues after extract injection is of intriguing importance. It is to be remembered that the immature rats used in the above experiments were usually fasted for six or twelve hours before being sacrificed. At the end of such periods the upper gastro-intestinal tract in these very small animals was usually empty. More puzzling was the fact that adrenalec-

tomized cats which had refused to take food for 24 to 48 hours also showed greatly augmented glucose and glycogen values after treatment with the extract (see following paper). The relatively small decreases which occur

TABLE 5

Carbohydrate values in normal young rabbits of 500–800 grams body weight

Animals starved 17 hours; injected with 3 cc. of extract, or adrenalin (1:2,000,000), or normal saline, per 100 grams body weight; killed 3 hours after injection.

RABBIT NUMBER	MATERIAL INJECTED	MUSCLE GLYCOGEN	LIVER GLYCOGEN	BLOOD SUGAR
		per cent	*per cent*	*mgm. per cent*
1	Saline	0.413	0.337	102
2	Saline	0.406	0.323	105
3	Adrenalin	0.314	0.303	121
4	Adrenalin	0.312	0.317	126
5	Extract	0.441	0.937	127
6	Extract	0.445	0.953	132
7	Extract	0.488	0.766	134
8	Extract	0.412	0.870	143
9	Extract	0.330	0.896	129

TABLE 6

Average levels of glucose and glycogen in different experimental groups

MATERIAL INJECTED	NUMBERS OF TABLES AVERAGED	TOTAL NUMBER OF ANIMALS USED	MUSCLE GLYCOGEN	LIVER GLYCOGEN	BLOOD SUGAR
Rats					
			per cent	*per cent*	*mgm. per cent*
Extract	1	10	0.463	0.78	132
Extract	2, 3	22	0.498	1.03	149
Extract	1, 2, 3	32	0.489	0.95	144
Adrenalin	1, 2, 3	14	0.246	0.31	110
Saline	1, 2, 3	10	0.297	0.32	87
None (non-fasting)	4	6	0.466	1.05	113
Rabbits					
Extract	5	5	0.427	0.89	133
Adrenalin	5	2	0.313	0.31	124
Saline	5	2	0.410	0.33	104

in blood lactates do not appear to be of particular significance, and other possible explanations are now being investigated.

Cortico-adrenal extract and hence by analogy the secretion of the adrenal cortex are thus shown to be of undoubted and far-reaching importance

in the regulation of carbohydrate metabolism. A discussion of the foregoing and other related and confirmatory observations appears in the following paper.

SUMMARY

Extracts of the adrenal cortex of proven potency have a marked influence on carbohydrate metabolism in normal animals (cats, rats, rabbits).

Young animals are particularly responsive subjects. In them the blood glucose and liver and muscle glycogen values are considerably elevated within an hour after injection of the extract. Six hours after injection (in rats) the levels are still maintained higher than in controls treated with adrenalin or saline solutions.

Storage of glycogen in the liver after extract treatment is enhanced three to five times above that found in the controls. This appears to represent a highly important function of the cortico-adrenal hormone.

Large amounts of dilute adrenalin solution (1:2,000,000), given intraperitoneally to young rats, produced increases in blood sugar and reductions in muscle glycogen. Liver glycogen was also in many cases reduced.

The increments in hepatic glycogen produced by the extract form the basis of a relatively simple and economical means of testing the potency of the material.

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THE APPARENT PREPOTENT FUNCTION OF THE ADRENAL GLANDS[1]

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Although the adrenal glands were brought forcefully to the attention of medical men about eighty years ago (Addison, 1855), and much speculation and patient inquiry have been devoted to them since that time, their functional significance has not yet been fully disclosed. Mass attacks by recent investigators have nevertheless circumscribed the adrenal problem more precisely, and accumulating evidence indicates the direction in which solution of the enigma may be found. Results which have been secured in this laboratory appear to justify at this time a consideration of the chief function of the adrenal tissues.

The Harvard school of workers have in the past decade presented a volume of reports on the activities of the adrenal medulla, and almost universal support of their findings has been forthcoming (Cannon, 1929). The emergency theory of adrenal function which has been put forward offers an admirable interpretation of the mode of medulliadrenal activity, and it appears now to be established. But knowledge of cortical function has been shrouded in conjecture up to the present time. That the cortex in contrast to the medulla is of premier importance in bodily economy has nevertheless been long appreciated. The fatal outcome which is observed a few days after adrenal extirpation is due specifically to cortical loss; furthermore, removal of the medulla along with other chromaphil (paraganglial) tissues in the body does not result in death (see review, Britton, 1930).

Preliminary results of experiments on the nature of cortico-adrenal activity which were begun here about three years ago suggested an important relationship to the metabolism of carbohydrates, and further experiments have strongly supported this idea (Britton *et al.*, 1931).

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Testimony to the profound and possibly primary involvement of the adrenal cortex in the regulation of carbohydrate metabolism is now presented.

METHODS. Studies have been made on liver and muscle glycogen, blood sugar and lactic acid values in *a*, untreated; *b*, extract-treated; *c* adrenalin-injected, and *d*, glucose-injected adrenalectomized cats. An

TABLE 1

Conditions in adrenalectomized animals showing symptoms within 48 hours of operation

CAT NUMBER	DATE OPERATED	SURVIVAL PERIOD	CONDITION OF ANIMAL WHEN KILLED	MUSCLE GLYCOGEN	LIVER GLYCOGEN	BLOOD SUGAR	LACTIC ACID
		hours		*per cent*	*per cent*	*mgm. per cent*	*mgm. per cent*
685	11/24/31	19	Very weak	0.223	0.254	39	28
701	12/ 5/31	48	In convulsions	0.380	0.121	55	17
702	12/ 5/31	47	Weak	0.358	0.133		37
703	12/ 7/31	18	Prostrated	0.194	0.179	43	48
709	1/ 4/32	26	In convulsions	0.444	0.188	64	14
710	1/13/32	47	In convulsions	0.547	0.130	43	53
711	1/14/32	24	Prostrated	0.470	0.142	39	
713	1/25/32	38	Prostrated	0.122	0.294	46	76
717	2/ 2/32	18	In convulsions	0.470	0.225	51	
720	2/ 3/32	28	Very weak	0.315	0.253	49	

TABLE 2

Conditions in untreated adrenalectomized cats showing symptoms of insufficiency 2–8 days after operation

CAT NUMBER	DATE OPERATED	CONDITION OF ANIMAL WHEN KILLED	MUSCLE GLYCOGEN	LIVER GLYCOGEN	BLOOD SUGAR	LACTIC ACID
			per cent	*per cent*	*mgm. per cent*	*mgm. per cent*
605	5/27/31	Moderately weak	0.268	0.095	55	38
606	5/28/31	In extremis	0.195	0.091	36	37
626	9/25/31	Weak		0.061	60	
627	9/26/31	Prostrated		0.067	46	23
629	10/ 2/31	Very weak	0.261	0.039		50
631	10/ 3/31	Weak	0.198	0.043		
632	10/ 2/31	Very weak	0.184	0.037		50
633	10/ 3/31	Moderately weak	0.231	0.048		
639	10/ 8/31	In convulsions	0.189	0.084	33	24
640	10/ 8/31	In convulsions	0.140	0.106	32	23

extended series of control experiments has also been carried out on unoperated animals. Two groups of experiments were also performed on adrenalectomized rats.

In the untreated series (cats) different degrees of adrenal insufficiency were allowed to develop, as noted in tables 1 and 2, before the animals were sacrificed by stunning and the tissues analyzed. Similarly in the extract-treated cases, insufficiency symptoms were first allowed to set in (tables

3 and 4), and thereafter the injections were started. Indications of insufficiency (refusal of food, weakness, prostration, convulsions) were generally observed from two to six days after adrenal removal by the one-stage operation. Their appearance indicated that a thorough operation had been performed, although necropsy was carried out when this seemed

TABLE 3

Carbohydrate values in adrenalectomized cats partially restored with cortico-adrenal extract

The animals showed general recovery but did not eat after extract was given.

CAT NUMBER	DATE KILLED	MUSCLE GLYCOGEN	LIVER GLYCOGEN	BLOOD SUGAR	LACTIC ACID
		per cent	*per cent*	*mgm. per cent*	*mgm. per cent*
648	10/23/31	0.539	0.187	86	41
653	10/30/31	0.359	0.409	112	36
661	10/30/31	0.600	0.489	109	31
662	10/30/31	0.503	0.250	86	39
665	11/ 1/31	0.469	0.324	86	36
666	11/17/31	0.323	0.385	95	30

TABLE 4

Carbohydrate values in adrenalectomized cats completely restored with cortico-adrenal extract

In all cases the animals attempted to take food after recovery.

CAT NUMBER	DATE KILLED	MUSCLE GLYCOGEN	LIVER GLYCOGEN	BLOOD SUGAR	LACTIC ACID
		per cent	*per cent*	*mgm. per cent*	*mgm. per cent*
683*	11/16/31	0.740	1.13	93	24
684*	11/17/31	0.373	1.53	73	28
704	12/15/31	0.530	0.66	103	34
705	12/16/31	0.740	1.25	106	22
706*	12/17/31	0.445	1.78	97	24
707	12/24/31	0.681	1.43	112	
712	1/22/32	0.433	0.52	142	29
714	1/28/32	0.337	0.64		
715	1/31/32	0.401	0.71	88	
716	1/31/32	0.565	0.59	82	
718	2/ 4/32	0.538	0.82	100	
722	2/ 5/32	0.574	0.81	117	

* Cat ate about 5 grams of salmon before it was killed.

necessary. Different amounts of extract were administered, usually from 20 to 50 cc. in 12 to 48 hours, according to the severity of the symptoms noted in each case.

The extract of the adrenal cortex used was prepared in this laboratory according to a modified Swingle-Pfiffner method (Britton and Silvette,

1931); this extract had previously been found to be potent in cases of complete adrenal insufficiency. One cubic centimeter of the material was equivalent to 40 grams of fresh whole adrenal glands. Recovery was well established in one or two days after starting extract treatment, although there were often signs of improvement within a few hours. In one group the disappearance of general symptoms alone was taken as indicative of recovery (table 3); in a second series the restoration of normal vigor and a definite disposition to take food were the significant criteria used (table 4). In the two series the animals were thus killed at different stages, and the tissues immediately taken for analysis.

Determinations of adrenalin and glucose effects were made on cats observed from two to four days after removal of the adrenals. The animals were apparently in good condition at this time. Adrenalin was given in dilution (1:2,000,000) equal to that found in the cortico-adrenal extract employed in series *b*, and also in similar amount. Different doses of glucose were also tested—usually from 30 to 50 cc. of a 5 or 10 per cent solution.

In all cases injections were given intraperitoneally in the present investigation, although the extract has been proved effective by mouth when given in large amounts (Britton, Flippin, and Silvette, 1931). The blood sugar method of Folin and Malmros (1929) and the lactic acid method of Friedemann, Cotonio and Shaffer (1927) were used. Determinations of liver and muscle glycogen were made according to a modification of Pflüger's method as described in the preceding paper (Silvette and Britton, 1932). Duly careful precautions were observed in removing all tissues; it was moreover first established that a standardized, rapid procedure allowed comparable determinations to be carried out.

Results (*cats*). Adrenalectomized animals were observed to suffer progressively severe derangements in carbohydrate metabolism following the operation (tables 1 and 2). The glucose in the blood and the glycogen in the liver became profoundly reduced, the latter sometimes to almost the disappearing point; even when the animals showed typical symptoms of adrenal insufficiency as early as 18 to 48 hours after operation, there were found considerable reductions below the normal levels. Muscle glycogen was also greatly diminished, while the blood lactates were somewhat increased.

The carbohydrate changes which we observed may be considered as revolutionary enough in themselves to bring about death of the animals. The reductions which occurred in the blood sugar (commonly to the convulsive level) and hepatic glycogen appear to be of greatest importance. Significantly, however, the glycogen content of heart muscle was found in several cases to be unaffected.

The administration of extract of the adrenal cortex to animals with symptoms of adrenal insufficiency produced a remarkably reversed picture

of that noted above. The liver and muscle glycogen and blood sugar levels were found to be increased, and the lactic acid values were concurrently decreased, to the normal limits (tables 3 and 4). The chemical changes took place in coincidence with the return of the animal to normal activity. In adrenalectomized animals which were obviously in a terminal dying condition, and which were undoubtedly suffering in a crucial degree from carbohydrate depletion, restoration of normal values was brought about by the extract (fig. 1).

It will be noted that the foregoing observations were made under various conditions on different series of animals. This was found necessary because of the inherent difficulties of operating on adrenalectomized

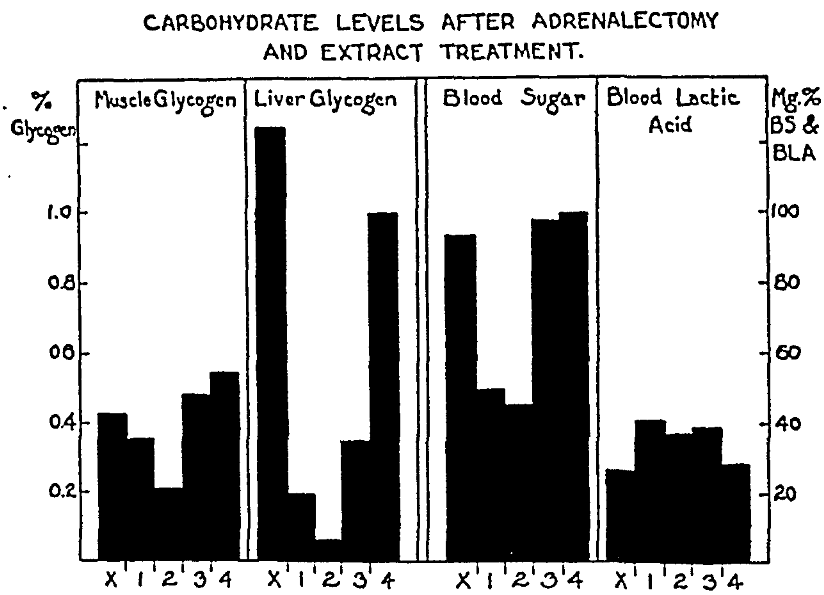

Fig. 1. *X*, Carbohydrate levels in normal cats; *1* and *2*, in adrenalectomized animals showing symptoms of insufficiency; *3* and *4*, after partial and complete restoration by extract (see text and table 6).

animals when symptoms were present. In a series of eight cases of adrenal insufficiency, however, tissue samples were taken for analysis when the animals were comatose, and attempts then made to restore them with cortical extract. Small amounts of ether or amytal were borne very poorly by such animals, and it was considered that the anesthetic probably vitiated the results. Administration of large amounts of extract nevertheless brought about recovery in some instances, i.e., after operation under ether, the dissection of muscle and liver samples, and withdrawal of about 3 cc. of blood from the heart. In these cases there occurred moderate glucose and glycogen increases.

It was significant, in those animals in which partial recovery only (disappearance of general symptoms of weakness, but no manifest return of

a keen interest in food) had been brought about, that there also occurred considerable increases in the blood sugar to the normal levels (table 3). Such increases took place in other experiments, indeed, within two to four hours after the extract had been administered (Britton and Silvette, 1931). The correlated increments in muscle and liver glycogen were also of notable extent.

It should be observed that the carbohydrate changes following extract injection were brought about in animals which had been in the fasting (anorexic) condition for 24 to 48 hours. Except in the three cases noted (table 4), in which a few grams of canned salmon were eaten, no food was allowed to be ingested by the animals after extract had been given. Food was presented to the animals merely as one of the tests of recovery.

Results in adrenalectomized rats. The following condensed protocol is indicative of the results obtained with cortico-adrenal extract on adrenalectomized rats:

Rats 114, 115, 116.

February 29, 1932, both adrenals excised, all cases.

March 9, animals appear well; food removed 4 p.m.

March 10, 1 p.m., gave 6 cc. per 100 grams body weight, of extract 117, to rats 114 and 115; gave 6 cc. saline per 100 grams to rat 116.

At 4 p.m., rats killed and blood and tissues taken for analysis.

Results:

RAT NUMBER	MATERIAL INJECTED	MUSCLE GLYCOGEN	LIVER GLYCOGEN	BLOOD SUGAR
		per cent	*per cent*	*mgm. per cent*
114	Extract	0.420	0.643	131
115	Extract	0.510	0.871	107
116	Adrenalin	0.148	0.295	90

In a further series of 24-hour fasted rats, adrenalectomized six days previously, the liver glycogen values were:

Extract-treated animals (6 cases)—4.74, 2.32, 1.34, 0.81, 0.64 and 0.54 per cent; adrenalin-injected (2 cases)—0.17, 0.18 per cent.

Effects of adrenalin and glucose. It is true that practically all our cortical extracts (and we have now made over 120 batches of 100 to 200 cc. each) give evidence that they contain adrenalin in small quantities. Although chiefly or primarily it promotes glycogenolysis in tissues, adrenalin is considered by some investigators to bring about in some way, possibly indirectly, an augmentation in liver glycogen (Cori, 1931). We have not found the latter true in our own experiments. Adrenalin does not, furthermore, restore adrenalectomized animals; it may bring about a slight, temporary amelioration of the symptoms which supervene after operation (Britton and Silvette, 1931). Animals with adrenal insufficiency which were given adrenalin in similar dosage and dilution to that found in cortico-

adrenal extract, and sacrificed from 1 to 10 hours afterward, showed only slightly higher levels of blood glucose and liver glycogen than those in the untreated series showing insufficiency symptoms (table 5-A). The muscle glycogen levels were observed to be somewhat higher than in the untreated cases. The effects were not at all comparable in magnitude to those observed after cortical extract administration.

The temporary restorative effect of glucose on adrenal insufficiency has been noted in our earlier papers (Britton, 1930; Britton and Silvette, 1931). Such restoration is accompanied by greatly augmented blood glucose

TABLE 5

Conditions in adrenalectomized, symptomless animals treated with adrenalin or glucose 2-4 days after operation, and in normal glucose-treated controls

EXPERIMENTAL CONDITIONS	CAT NUMBER	DATE	MUSCLE GLYCOGEN	LIVER GLYCOGEN	BLOOD SUGAR	LACTIC ACID
			per cent	*per cent*	*mgm. per cent*	*mgm. per cent*
A. Adrenalectomized; adrenalin treated	666	11/ 6/31	0.462	0.215	46	21
	667	11/ 6/31		0.212	43	44
	668	11/ 6/31	0.314	0.276	53	36
	669	11/ 9/31	0.391	0.231	50	33
	680	11/18/31	0.400	0.217	64	18
B. Adrenalectomized; glucose treated	682	11/19/31			295	*
	688	12/ 2/31	0.396	0.430	318	25*
	689	12/ 2/31	0.342	0.154	274	*
	690	12/ 2/31	0.304	0.165	278	*
	691	12/ 7/31	0.498	0.349	167	69*
C. Normal controls; glucose treated	693	11/30/31	0.301	2.720	97	22
	694	11/30/31	0.450	1.945	73	21
	695	12/ 3/31	0.514	2.215	81	30
	696	12/ 3/31	0.344	3.41	101	28
	697	12/ 9/31	0.280	1.31	218	*
	698	12/ 9/31	0.309	1.19	250	*

* Determinations vitiated by large amounts of glucose present in blood samples.

levels, as well as small increases in muscle glycogen. The ability of adrenalectomized animals to store liver glycogen under heavy glucose dosage is nevertheless observed to be greatly reduced; normal animals treated similarly with glucose stored in contrast from eight to ten times as much. Furthermore, adrenalectomized animals treated with extract, but *without* glucose, built up from four to five times the amount of liver glycogen stored by similar experimental animals which were given glucose without extract (tables 5 and 6).

Effects on carbohydrate metabolism of various operative procedures. For

purposes of comparison we have made analyses of the glycogen and blood sugar values in a small number of pancreatectomized (diabetic) and functionally hepatectomized animals. The effects of adrenalectomy on diabetic animals have also been noted.

Completely pancreatectomized cats showed blood sugar levels of about 200 to 300 mgm. per 100 cc. within a few days after operation (sometimes they reached 600 mgm.), and there was a considerable associated loss of body weight. When the animals were very weak and emaciated, a week or ten days after operation, they were sacrificed and their tissues analyzed. Liver glycogens were found to be about 400 mgm. and muscle glycogens about 450 mgm. per 100 grams of tissue.

TABLE 6

Average carbohydrate levels in animals under different experimental conditions

TABLE NUMBER	NUMBER OF CATS IN SERIES	EXPERIMENTAL CONDITIONS	MUSCLE GLYCOGEN	LIVER GLYCOGEN	BLOOD SUGAR	BLOOD LACTIC ACID
			per cent	*per cent*	*mgm. per cent*	*mgm. per cent*
X*	11	Normal, fasting 24 hours	0.426	1.22	92	25
1	10	Adrenalectomized, symptoms within 48 hours	0.352	0.192	48	39
2	10	Adrenalectomized, symptoms in 2–6 days	0.208	0.067	44	35
3	6	Adrenalectomized, partly restored	0.466	0.341	96	37
4	12	Adrenalectomized, restored by extract	0.530	0.990	98	27
5-A	5	Adrenalectomized, symptoms, adrenalin injected	0.392	0.230	51	31
5-B	5	Adrenalectomized, symptoms, glucose treated	0.385	0.275	266	
5-C	6	Unoperated, glucose treated	0.366	2.13	137	25

* See table 2, Silvette and Britton. 1932.

Removal of the adrenals from completely diabetic animals resulted notably in an early and marked fall in the blood-sugar level. The liver and muscle glycogen values were lower than in those cases of pancreatectomy alone. Pancreatico-adrenal relationships are now being further investigated.

In six young cats, blood sugar and muscle glycogen samples were taken under ether, and the liver was then practically excluded from the circulation by tying off the vessels in the hepatic pedicle (three cases) and also the abdominal aorta and vena cava (three cases). When the animals were observed to be prostrated within a few hours after operation, they were killed and examined. The muscle glycogen levels were found to have been

reduced to 150 to 250 mgm. per cent, while the blood samples sometimes showed hypoglycemic values.

In pancreatectomy and in (functional) hepatectomy, therefore, the carbohydrate values were no more severely affected than in cases of adrenal insufficiency.

The development of adrenal insufficiency and recovery by cortical extract. We have found the following conditions to be characteristic of experimental adrenal insufficiency: General symptoms—loss of weight, refusal of food, weakness, prostration, convulsions; decreases in body temperature, blood sugar, liver and muscle glycogen; increases in blood concentration, blood lactic acid and non-protein nitrogen, and serum calcium and phosphorus. The recovery of prostrate adrenalectomized animals with cortico-adrenal extract, though obviously gradual and progressive, may well be divided according to our observations into two phases as follows:

Earliest signs of recovery

Disappearance of insufficiency symptoms
Increase in blood sugar
Increase in body temperature
Decrease in blood concentration

Secondary signs of recovery

Increase in liver glycogen
Increase in muscle glycogen
Decrease in blood lactic acid
Decrease in blood non-protein nitrogen
Decrease in serum calcium
Decrease in serum phosphorus
Reappearance of normal appetite
Gain in weight

In earlier papers we have reported on changes in blood cell volume and non-protein nitrogen in adrenal insufficiency and after extract administration. Recently we have also followed the variations in blood cell counts, and in serum calcium and phosphorus. None of these changes appears to us at present, however, to be of primary importance in connection with cortico-adrenal function. Rather the profound carbohydrate changes appear to be of the greatest significance.

Discussion. Investigators at Princeton and Johns Hopkins (Harrop *et al.*, 1931) have attributed considerable importance to the marked changes in renal secretion which follow adrenalectomy, and the amelioration which is brought about by the administration of cortico-adrenal extract. The augmentation of blood urea in adrenal insufficiency, and the reduction effected by the extract, are also believed by them to be of outstanding significance. In a previous report (Britton and Silvette, 1931) we have pointed out that such effects are probably indirect or secondary to more fundamental changes.

It can hardly be considered that the accumulation of unutilizable protein end-products in the body, or the diminution in kidney secretion, represent pre-eminently critical conditions in adrenal insufficiency. Nor can it be reasonably entertained that the precocious sexual developments which we have observed to be brought about by cortical extract (Corey and Britton, 1931), interestingly significant though they may be, reflect the primary activity of the adrenal cortex. That the effects on the sexual organs probably indicate the activity of a hormone of a second-class order which is present in the crude extract has been postulated.

The activities of many of the glands of internal secretion appear to be more or less intimately connected with carbohydrate metabolism. In the case of the pancreas as well as the adrenals this is particularly true. Substances derived from these organs may bring about extraordinary shifts in carbohydrate balance in the organism. In an excellent article by Cori (1931) the emphasis is thrown almost equally on insulin and adrenalin as factors in regulating carbohydrate metabolism. That the cortex of the adrenal glands may be specifically concerned with the storage and utilization of carbohydrates in the body was foreshadowed by one of us in a recent review (Britton, 1930).

About twenty years ago Biedl (1913) concluded from collected data that the adrenals were related to carbohydrate metabolism. From table 7 it will be observed that many investigators have noted diminutions in blood sugar and liver glycogen (checked ✓) in different animals following adrenalectomy. After rather extensive blood-chemical analyses, Swingle (1927) concluded that death after adrenal removal may possibly be due to either of two causes, hypoglycemia (first) or acid intoxication. Rogoff and Stewart (1918, 1926) have vigorously contended, however, that the fall in blood sugar is related to the terminal symptoms of insufficiency. That the glycemic changes are terminal expressions has also been maintained by Banting and Gairns (1926).

It is apparent from our own experiments that progressively severe reductions in the blood glucose and liver glycogen levels occur in adrenalectomized, untreated animals. Muscle glycogen is also considerably reduced, while the blood lactates are increased. Such changes do not follow when the adrenal medulla alone is extirpated. Many weeks after medulliadrenalectomy, indeed, the blood glucose and the liver and muscle glycogen percentages may be quite normal (Britton, Geiling and Calvery, 1928).

It is to be noted also in comparison that even in the severest exhausting conditions, and in inanition and exposure of animals to cold, in death from insulin or strychnine convulsions and even in experimental diabetes, the hepatic and muscle glycogen values are not often reduced beyond the low levels which we have observed in adrenalectomized animals. Moreover, the muscle glycogen and blood glucose in cases of hepatectomy are

not depleted more thoroughly than in animals showing more or less severe symptoms of adrenal insufficiency. And in hepatectomy (Bollman, Mann and Magath, 1925) as well as in pancreatectomy death is admitted to be due primarily to carbohydrate deficiencies (see also Chaikoff, 1927).

Animals which are dying from pancreatectomy or from parathyroidectomy may be restored by hormone-containing extracts of the respective glands. Thus the evidence points to the conclusion and it is generally recognized that insulin and parathyroid extract are primarily regulators of carbohydrate and calcium balance respectively in the organism. The experiments now cited offer proof that animals dying after adrenal extir-

TABLE 7

Investigations by various authors on adrenalectomized animals

Diminutions in blood glucose and liver glycogen were observed as indicated by check mark (√).

AUTHOR	DATE	ANIMALS USED	BLOOD SUGAR DECREASED	LIVER GLYCOGEN DECREASED
Bierry and Malloizel	1908	Dogs	√	*
Porges	1909–10	Dogs	√	√
Mackenzie	1917	Dogs	*	√
Estrada	1926	Dogs	√	√
Swingle	1927	Dogs, cats	√	*
Hartman	1927	Cats	√	*
Schwarz	1910	Rats	*	√
Kahn and Stark	1911	Rats	*	√
Kuriyama	1918	Rats	√	√
Artundo	1927	Rats	√	√
Cori	1927	Rats	√	√
Houssay and Artundo	1929	Rats	*	√
Wyman and Walker	1929	Rats	√	*
Lumley and Nice	1930	Rats	√	*

In the starred (*) cases no analyses were reported. Muscle glycogen was found by a few authors to be reduced.

pation show profoundly reduced carbohydrate levels, quite sufficient indeed to produce death; and also that the hormone-containing extract of the adrenal cortex brings about ready restoration through its effect in raising notably the blood glucose and liver glycogen. In normal animals, too, cortical extract induces very considerable increments in carbohydrate values.

It would thus appear that the adrenal cortex is indispensably important in maintaining, in coöperation with other organs, the normal metabolism of carbohydrates in the body. The evidence indicates that this represents the principal function of the cortico-adrenal tissues. The results appear significant in view of the severe myasthenic condition found in Addison's

disease, and also in relation to the recently observed effects of cortico-adrenal extract in stimulating energy output (Eagle and Britton, 1932).

SUMMARY

The effects of adrenalectomy on carbohydrate metabolism are profound in character and appear to be primarily responsible for death of the animal. Blood glucose and liver glycogen are chiefly affected; these show marked reductions from the normal levels. There are also associated decreases in muscle glycogen, and increases in blood lactates. The glycogen of heart muscle is not reduced.

In pancreatectomized and in hepatectomized animals, carbohydrate metabolism is affected in no more critical degree than in cases of experimental adrenal insufficiency.

The glycogenic and glycemic changes which occur after complete adrenalectomy are not observed in cases of extirpation of the adrenal medulla alone.

Administration of cortico-adrenal extract brings about recovery of animals which are in the terminal stages of insufficiency, apparently through initial restoration of normal carbohydrate values. Increments in circulating sugar to the normal or even to hyperglycemic levels occur early after extract injection and are invariably associated with the disappearance of insufficiency symptoms. There are also correlated and striking increases in liver glycogen. These conditions appear to be of primary importance in effecting recovery. Restoration is completed with the re-establishment of muscle glycogen values and the diminution of blood lactic acid.

Cortico-adrenal extract increases the blood sugar and the liver and muscle glycogen values in normal (particularly young) animals (see preceding paper).

None of the results are produced by the small amount of adrenalin (1:2,000,000) contained in the extract.

Completely adrenalectomized animals show a markedly reduced ability to store liver glycogen. Normal individuals which were injected with glucose store from eight to ten times as much glycogen as operated animals.

In the light of present as well as earlier evidence from this laboratory, it is indicated that the cortico-adrenal tissues and their pertinent hormone as contained in our extracts are primarily concerned (in conjunction admittedly with other secretions) in the maintenance of normal glucose and glycogen levels in the body. Since the cortex represents that part of the organs which is pre-eminently essential to life, the conclusion is derived that the regulation of carbohydrate metabolism may be considered as the prepotent function of the adrenal glands.

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